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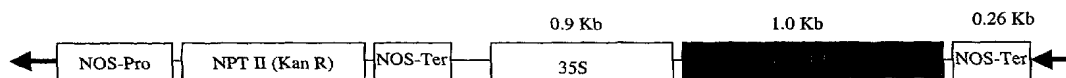
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(54) Title: COMPOSITIONS AND METHODS OF INCREASING STRESS TOLERANCE IN PLANTS



(57) Abstract: The present invention provides novel isolated FT polynucleotides and polypeptides encoded by the FT polynucleotides. Also provided are the antibodies that immunospecifically bind to a FT polypeptide or any derivative, variant, mutant or fragment of the FT polypeptide, polynucleotide or antibody. The invention additionally provides methods of constructing transgenic plants that have altered levels of FT polynucleotides and polypeptides.



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COMPOSITIONS AND METHODS OF INCREASING STRESS TOLERANCE IN PLANTS

FIELD OF THE INVENTION

5 The invention relates in part to novel plant farnesyl transferase alpha and beta subunit polynucleotides and polypeptides. Also included are transgenic plants expressing the novel polynucleotides and polypeptides. The invention also includes transgenic plant cells, tissues and plants having novel phenotypes resulting from the expression of these polynucleotides in either the sense or antisense orientation.

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BACKGROUND OF THE INVENTION

Most higher plants encounter at least transient decreases in relative water content at some stage of their life cycle and, as a result, have evolved a number of desiccation protection mechanisms. If however, the change in water deficit is prolonged the effects on
15 the plants growth and development can be profound. Decreased water content due to drought, cold or salt stress can irreparably damage plant cells which in turn limits plant growth and crop productivity in agriculture.

Plants respond to adverse conditions of drought, salinity and cold with a variety of morphological and physiological changes. Although our understanding of plant tolerance
20 mechanisms to these stresses is incomplete, the plant hormone abscisic acid (ABA) is believed to be an essential mediator between environmental stimulus and plant responses. ABA levels increase in response to water deficits and exogenously applied ABA mimics many of the responses induced by water-stress. Once ABA is synthesized it causes the closure of the leaf stomata thereby decreasing water loss through transpiration.

25 The identification of genes that transduce ABA into a cellular response opens the possibility of exploiting these regulators to enhance desiccation tolerance in crop species. In principle, these ABA signaling genes can be coupled with the appropriate controlling elements to allow optimal plant growth, development and productivity. Thus, not only would these genes allow the genetic tailoring of crops to withstand transitory
30 environmental stresses, but they should also broaden the environments where traditional crops can be grown.

The recent isolation of an *Arabidopsis thaliana* mutant, *eral*, is hypersensitive to ABA and has been shown to also be tolerant to conditions of water deprivation. ERA1 has been identified as a β subunit of farnesyl transferase. Farnesyl transferase is a heterodimeric enzyme that provides the specific addition of a farnesyl pyrophosphate moiety onto the substrate target sequence. The target sequence is defined as a sequence of four amino acids which are present at the carboxy terminus of the protein and is referred to as a CaaX motif in which the "C" is cysteine, "a" is any aliphatic amino acid and "X" is any amino acid. The α subunit is common with a second prenylation enzyme, geranylgeranyl transferase, that has a different β subunit and adds a geranylgeranyl isoprenyl pyrophosphate moiety to the target sequence.

Prenylation is a multistep pathway which includes prenylation of the cysteine residue of the CaaX site, cleavage of the -aaX tripeptide and methylation of the prenyl-cysteine residue. Potentially, each of these steps could represent a target for genetic manipulation of the prenylation process to generate a desired phenotype such as stress tolerance.

In plants, prenylation has been linked to cell cycle control, meristem development, and phytohormone signal transduction, however, few details of the role of prenylation, the substrate proteins or the extent to which the plant system will be analogous to the mammalian and yeast systems are known. The most characterized substrates for CaaX modification are the Ras and a-factor proteins of yeast. Although there are three steps to complete protein maturation, abolition or modification of any one step does not necessarily result in cessation of target biological activities. Ras function is attenuated if the -aaX tripeptide is not cleaved but not abolished and some proteins retain the -aaX tripeptide after farnesylation. These observations may be substrate specific as, in contrast, there are examples indicating some proteins are fully functional only after being properly prenylated such as in regulating processes such as mitogen response in mammals and mating pheromone in yeast.

In *Arabidopsis thaliana*, more than 600 proteins contain a CaaX motif, suggesting a role for the post-translational modification by prenylation in numerous cellular processes. In *Arabidopsis thaliana*, it has been demonstrated that the loss-of-function of the β -subunit of farnesyl transferase will result in a ABA-hypersensitive phenotype. Although it is still not clear why plants lacking the functional β -subunit of farnesyl transferase become more sensitive to ABA, it clearly suggests that protein prenylation is

involved in regulation of the homeostasis of ABA sensitivity. The balance of ABA cellular responses, whether more sensitive or less sensitive to ABA, is possibly regulated by the relative activities of prenylated proteins.

This invention is directed at the manipulation of the farnesyl transferase (FT) subunits, either α or β (FTA, FTB) to alter farnesyl transferase enzyme expression and activity. Farnesyl transferase catalyses the first step of farnesylation in which a 15-carbon farnesyl moiety is added to the cysteine residue of the target sequence CaaX. Included in this invention are vector constructs containing FTA or FTB sequences under the control of appropriate regulatory sequences to produce phenotypes such as, but not limited to, water-stress tolerance, increased biomass accumulation, increased yield or delayed senescence. Manipulation of the FTA subunit may also affect the activity of geranylgeranyl transferase and the phenotypes associated with this manipulation are encompassed by this invention.

SUMMARY OF THE INVENTION

The present invention is based in part upon the discovery of novel farnesyl transferase nucleic acid sequences and polypeptides from *Arabidopsis thaliana*, *Brassica napus*, *Glycine max* and *Zea maize*. The nucleic acids, polynucleotides, proteins and polypeptides, or fragments thereof described herein are collectively referred to as FT nucleic acids and polypeptides.

Accordingly, in one aspect, the invention provides an isolated nucleic acid molecule that includes the sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, or a fragment, homolog, analog or derivative thereof. The nucleic acid can include, *e.g.*, a nucleic acid sequence encoding a polypeptide at least 99% identical to a polypeptide that includes the amino acid sequences of SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36, or SEQ ID NO:37, a nucleic acid sequence encoding a polypeptide at least 85% identical to a polypeptide that includes the amino acid sequences of SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9 or a nucleic acid sequence encoding a polypeptide at least 99% identical to a polypeptide that includes the amino acid sequences of SEQ ID NO:33, SEQ ID NO:36, or SEQ ID NO:39. The nucleic acid can be, *e.g.*, a genomic DNA fragment, or a cDNA molecule.

The invention also includes the nucleic acid sequences of SEQ ID NO: 2, 3, 4, 29, 30, 32, 35, 38, 40-57 or 58. Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors or nucleic

acids described herein. In some aspects the FT nucleic acid is operably linked to a promoter. Examples of promoter includes a constitutive promoter (e.g., 35S CaMV, MuA), an ABA inducible promoter (e.g., RD29A), tissue specific promoters (e.g., CUT1) or a guard cell-specific promoter (e.g., 35S, MuA and RD29A)

5 The invention is also directed to host cells transformed with a vector comprising any of the nucleic acid molecules described herein.

 The invention is also directed to plants and cells transformed with a FT nucleic acid or a vector comprising a FT nucleic acid. Also included in the invention is the seed, and progeny of the transformed plants or cells.

10 The invention is also further directed to the use of plants and cells transformed with a FT nucleic acid or a vector comprising a FT nucleic acid in generation of mutant libraries and genetic screening protocols.

 In a further aspect, the invention includes a substantially purified FT polypeptide, *e.g.*, any of the FT polypeptides encoded by an FT nucleic acid, and fragments, homologs, analogs, and derivatives thereof.

15 In still a further aspect, the invention provides an antibody that binds specifically to an FT polypeptide. The antibody can be, *e.g.*, a monoclonal or polyclonal antibody, and fragments, homologs, analogs, and derivatives thereof. The invention is also directed to isolated antibodies that bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above.

 The invention also includes a method of producing a transgenic plant which has increased stress resistance such as, but not limited to, water deficit, or increased biomass, increased yield; delayed senescence or increases ABA sensitivity by introducing into one or more cells of a plant a compound that alters FT expression or activity in the plant. In one aspect the compound is a FT nucleic acid. The nucleic acid can be for example a inhibitor or farnesylation or genanylgerylation. Alternatively, the compound is a FT double stranded RNA-inhibition hair-pin nucleic acid or FT antisense nucleic acid.

25 The invention further provides a method for producing a FT polypeptide by providing a cell containing an FT nucleic acid, *e.g.*, a vector that includes a FT nucleic acid, and culturing the cell under conditions sufficient to express the FT polypeptide encoded by the nucleic acid. The expressed FT polypeptide is then recovered from the cell. Preferably, the cell produces little or no endogenous FT polypeptide. The cell can be, *e.g.*, a prokaryotic cell or eukaryotic cell.

The invention is also directed to methods of identifying a FT polypeptide or nucleic acid in a sample by contacting the sample with a compound that specifically binds to the polypeptide or nucleic acid, and detecting complex formation, if present.

5 The invention further provides methods of identifying a compound that modulates the activity of a FT polypeptide by contacting a FT polypeptide with a compound and determining whether the FT polypeptide activity is modified.

The invention is also directed to compounds that modulate FT polypeptide activity identified by contacting a FT polypeptide with the compound and determining whether the compound modifies activity of the FT polypeptide, binds to the FT polypeptide, or binds
10 to a nucleic acid molecule encoding a FT polypeptide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable
15 methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

20 Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration depicting the pBI121 antisense FTA vector construct.

Figure 2 is an illustration of genomic Southern hybridization analysis of anti-FTA
25 transgenic *Arabidopsis thaliana*.

Figure 3 is an illustration of Northern analysis of five 35S-anti-FTA *Arabidopsis thaliana* lines (T3 plants).

Figure 4 shows a Western expression analysis using anti-FTA antibodies to detect the FTA polypeptides.

30 Figure 5 is a set of photographs showing ABA effects on seedling growth and development. FTA Antisense transgenic seedlings exhibit enhanced ABA sensitivity.

Figure 6 shows the effect of ABA on seedling growth and development.

Figure 7 shows photographs of wild type Columbia (A) and four antisense FTA transgenic lines (B, C, D, E) of *Arabidopsis thaliana* after 8 days without watering.

Figure 8 is an illustration of the homology among FTA nucleic acid (A) and amino acid (B) sequences from various plant species based on ClustalW analysis (percent identity shown).

Figure 9 is an illustration of the homology among FTB nucleic acid and amino acid sequences from various plant species based on ClustalW analysis (percent identity shown).

Figure 10 is an illustration of transgenic performance during water stress.

Figure 11 is an illustration of shoot fresh weight, or biomass accumulation, after 6 days of water stress treatment and 6 days recovery time.

Figure 12 is an illustration of seed yield (grams) obtained under optimal conditions or following a 6 day water stress treatment.

Figure 13 is an illustration of vegetative growth under optimal conditions, shown is shoot fresh weight 6 days after the first flower opened.

Figure 14 is an illustration of the effect of a biotic stress coupled with drought stress treatment on seed yield.

Figure 15 is a representative illustration of gel electrophoresis analysis of PCR products in an assay to detect transgenic lines of *Brassica napus*.

DETAILED DESCRIPTION OF INVENTION

The present invention provides a novel farnesyl transferase (FT) nucleic acid sequences (SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37) and their encoded polypeptides (SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39) isolated from *Brassica napus* (Bn), *Arabidopsis thaliana* (At), *Glycine max* (Gm) and *Zea maize* (Zm). The sequences are collectively referred to as “FT nucleic acids” or FT polynucleotides” and the corresponding encoded polypeptide is referred to as a “FT polypeptide” or “FT protein”. Farnesyl transferase subunits, Alpha (α) and Beta (β) are referred to as FTA and FTB, respectively. *Glycine max* is also referred to as soy or soybean throughout the specification. *Zea maize* is also referred to as *Zea mays* or corn throughout the specification. These terms are interchangeable. Unless indicated otherwise, “FT” is meant to refer to any of the novel sequences disclosed herein.

Table A provides a summary of the FT nucleic acids and their encoded polypeptides.

TABLE A. Sequences and Corresponding SEQ ID Numbers

FT Assignment	Identification	SEQ ID NO (nucleic acid)	SEQ ID NO (polypeptide)
1	<i>Arabidopsis thaliana</i> farnesyl transferase alpha subunit	1	5
2	<i>Brassica napus</i> farnesyl transferase alpha subunit	6	7
3	<i>Brassica napus</i> farnesyl transferase beta subunit	8	9
4	<i>Glycine max</i> alpha subunit	31	33
5	<i>Glycine max</i> beta subunit	34	36
6	<i>Zea mays</i> beta subunit	37	39

Also included in the invention are nucleic acids that are complementary to the disclosed FT nucleic acid sequences. For example, SEQ ID NO: 2, 3, 29, 30, 32, 35 or 38. Further provided by the invention are constructs comprising FT antisense nucleic acid molecules as disclosed in for example SEQ ID NO: 4, 40-58.

Based on their structural and functional relatedness to known farnesyl transferase proteins, the FT proteins are novel members of the farnesyl transferase family of proteins. (See, Example 3) FT nucleic acids, and their encoded polypeptides, according to the invention are useful in a variety of applications and contexts. For example, the nucleic acids can be used to produce transgenic plants that have an increased resistance to biotic and abiotic stresses, *e.g.*, chilling stress, salt stress, heat stress, water stress, wound healing, pathogen challenge, or herbicides.

This invention includes methods to up-regulate the FT enzyme activity in transgenic plants, cells and tissue cultures by using an over-expression vector construct and methods to down-regulate the FT enzyme activity in transgenic plants, cells and tissue cultures by using a double stranded RNA-inhibition, hairpin vector construct. These methods are by way of example to produce the up-regulation or down-regulation effects and are not meant to be limiting as to the method of achieving this outcome.

Additionally, the nucleic acids and polypeptides according to the invention may be used as targets for the identification of small molecules that modulate or inhibit, FT activity. Alternatively, the FT nucleic acids and polypeptides can be used to identify proteins that are members of the farnesyl transferase family of associated proteins.

Further, the modulation or inhibition of FT activity may be achieved by modifications to the nucleic acid sequences of FTA or FTB by the actions of chemical mutagens or irradiation. Expression of FT nucleic acids which encode enzymatically non-

functional FT polypeptides can be used to evoke a dominant-negative inhibitory effect on FT activity.

Additional utilities for FT nucleic acids and polypeptides according to the invention are disclosed herein.

5 **FT Nucleic Acids**

The nucleic acids of the invention include those that encode a FT polypeptide or protein. As used herein, the terms polypeptide and protein are interchangeable.

In some embodiments, a FT nucleic acid encodes a mature FT polypeptide. As used herein, a “mature” form of a polypeptide or protein described herein relates to the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein. The product “mature” form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps that may take place within the cell in which the gene product arises. Examples of such processing steps leading to a “mature” form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a “mature” form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

Among the FT nucleic acids is the nucleic acid whose sequence is provided in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 or a fragment thereof. Additionally, the invention includes mutant or variant nucleic acids of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID

NO:34, or SEQ ID NO:37, or a fragment thereof, any of whose bases may be changed from the corresponding base shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, while still encoding a protein that maintains at least one of its FT-like activities and physiological functions. The invention further includes the complement of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, including fragments, derivatives, analogs and homologs thereof. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications.

One aspect of the invention pertains to isolated nucleic acid molecules that encode FT proteins or biologically active portions thereof. Also included are nucleic acid fragments sufficient for use as hybridization probes to identify FT-encoding nucleic acids (*e.g.*, FT mRNA) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of FT nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

"Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as about, *e.g.*, 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example,

in various embodiments, the isolated FT nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, or a complement of any one of the nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 as a hybridization probe, FT nucleic acid sequences can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to FT nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NO:1, SEQ ID NO:6, SEQ

ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37. For example, a complimentary nucleic acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:35 or SEQ ID NO:38. In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, thereby forming a stable duplex.

As used herein, the term “complementary” refers to Watson-Crick or Hoogsteen base pairing between nucleotide units of a nucleic acid molecule, and the term “binding” means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Von der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, *e.g.*, a fragment that can be used as a probe or primer, or a fragment encoding a biologically active portion of FT. Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids,

respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are
5 nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type.

Derivatives and analogs may be full length or other than full length, if the

10 derivative or analog contains a modified nucleic acid or amino acid, as described below.

Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, 85%, 90%, 95%, 98%, or even 99% identity (with a preferred identity of 80-99%) over a
15 nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR
20 BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below. An exemplary program is the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison, WI) using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482-489, which is incorporated herein by reference in its entirety).

25 A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of a FT polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA.

30 Alternatively, isoforms can be encoded by different genes. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33,

SEQ ID NO:36 or SEQ ID NO:39, as well as a polypeptide having FT activity, *e.g.* substrate binding.

The nucleotide sequence determined from the cloning of the *Arabidopsis thaliana* FT gene allows for the generation of probes and primers designed for use in identifying and/or cloning FT homologues in other cell types, *e.g.*, from other tissues, as well as FT homologues from other plants. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 or more consecutive sense strand nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37; or an anti-sense strand nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37; or of a naturally occurring mutant of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37.

Probes based on the *Arabidopsis thaliana* FT nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a FT protein, such as by measuring a level of a FT-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting FT mRNA levels or determining whether a genomic FT gene has been mutated or deleted.

A "polypeptide having a biologically active portion of FT" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of FT" can be prepared by isolating a portion of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 that encodes a polypeptide having a FT biological activity (biological activities of the FT proteins are described below), expressing the encoded portion of FT protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of FT. In another embodiment, a nucleic acid fragment encoding a biologically active portion of FT includes one or more regions.

FT Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 due to the degeneracy of the genetic code.

5 These nucleic acids thus encode the same FT protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, *e.g.*, the polypeptide of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a
10 protein having an amino acid sequence shown in SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39.

In addition to the *Arabidopsis thaliana* FT nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that
15 lead to changes in the amino acid sequences of FT may exist within a population (*e.g.*, the plant). Such genetic polymorphism in the FT gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a FT protein, preferably a plant FT protein. Such natural allelic variations can
20 typically result in 1-5% variance in the nucleotide sequence of the FT gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in FT that are the result of natural allelic variation and that do not alter the functional activity of FT are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding FT proteins from other species, and
25 thus that have a nucleotide sequence that differs from the sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the FT cDNAs of the invention can be isolated based on their homology to the *Arabidopsis thaliana* FT nucleic acids disclosed herein using the
30 cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID

NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500 or 750 nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent
5 conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding FT proteins derived from species other than *Arabidopsis thaliana*) or other related sequences (*e.g.*, paralogs) can be obtained by low,
10 moderate or high stringency hybridization with all or a portion of the particular sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in
15 different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence
20 hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at T_m , 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*,
25 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989),
30 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions is hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm

DNA at 65°C. This hybridization is followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 corresponds to a naturally occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA* 78: 6789-6792.

Conservative mutations

In addition to naturally-occurring allelic variants of the FT sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, 5 SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, thereby leading to changes in the amino acid sequence of the encoded FT protein, without altering the functional ability of the FT protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37. A 10 "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of FT without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the FT proteins of the present invention, are predicted to be particularly unamenable to alteration.

15 Another aspect of the invention pertains to nucleic acid molecules encoding FT proteins that contain changes in amino acid residues that are not essential for activity. Such FT proteins differ in amino acid sequence from SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence 20 encoding a protein, wherein the protein comprises an amino acid sequence at least about 75% homologous to the amino acid sequence of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39. Preferably, the protein encoded by the nucleic acid is at least about 80% homologous to SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39, more preferably at 25 least about 90%, 95%, 98%, and most preferably at least about 99% homologous to SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39.

An isolated nucleic acid molecule encoding a FT protein homologous to the protein of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 30 or SEQ ID NO:39 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in FT is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a FT coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for FT biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

In one embodiment, a mutant FT protein can be assayed for (1) the ability to form protein:protein interactions with other FT proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant FT protein and a FT receptor; (3) the ability of a mutant FT protein to bind to an intracellular target protein or biologically active portion thereof; (*e.g.*, avidin proteins); (4) the ability to bind FT protein; or (5) the ability to specifically bind an anti-FT protein antibody.

Antisense FT Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the

coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire FT coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a FT protein of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39, or antisense nucleic acids complementary to a FT nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding FT (*e.g.* SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37). The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the protein coding region of *Arabidopsis thaliana* FT corresponds to SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding FT (*e.g.* SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37). The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

In various embodiments the anti-sense FT nucleic acid molecule includes the sequences of SEQ ID NO: 2, 3, 29, 30, 32, 35 or 38.

Given the coding strand sequences encoding FT disclosed herein (*e.g.*, SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of FT mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of FT mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of FT mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense

nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w; and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a FT protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15:

6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA -DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in applications.

10 **Double Stranded RNA Inhibition (RNAi) by Hairpin Nucleic Acids**

Another aspect of the invention pertains to the use of post transcriptional gene silencing (PTGS) to repress gene expression. Double stranded RNA can initiate the sequence specific repression of gene expression in plants and animals. Double stranded RNA is processed to short duplex oligomers of 21-23 nucleotides in length. These small interfering RNA's suppress the expression of endogenous and heterologous genes in a sequence specific manner (Fire *et al.* *Nature* 391:806-811, Carthew, *Curr. Opin. in Cell Biol.*, 13:244-248, Elbashir *et al.*, *Nature* 411:494-498). A RNAi suppressing construct can be designed in a number of ways, for example, transcription of a inverted repeat which can form a long hair pin molecule, inverted repeats separated by a spacer sequence that could be an unrelated sequence such as GUS or an intron sequence. Transcription of sense and antisense strands by opposing promoters or cotranscription of sense and antisense genes.

FT Ribozymes and PNA moieties

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as a mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave FT mRNA transcripts to thereby inhibit translation of FT mRNA. A ribozyme having specificity for a FT-encoding nucleic acid can be designed based upon the nucleotide sequence of a FT DNA disclosed herein (*i.e.*, SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a FT-encoding

mRNA. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, FT mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

5 Alternatively, FT gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the FT (*e.g.*, the FT promoter and/or enhancers) to form triple helical structures that prevent transcription of the FT gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

10 In various embodiments, the nucleic acids of FT can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs"

15 refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols

20 as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

 PNAs of FT can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting

25 replication. PNAs of FT can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

30 In another embodiment, PNAs of FT can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of FT can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA

recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity.

PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996)

5 above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl) amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end
10 of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

15 FT Polypeptides

A FT polypeptide of the invention includes the protein whose sequence is provided in SEQ ID NO:5, SEQ ID NO:7, OR SEQ ID NO:9. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or
20 SEQ ID NO:39 while still encoding a protein that maintains its FT-like activities and physiological functions, or a functional fragment thereof. In some embodiments, up to 20% or more of the residues may be so changed in the mutant or variant protein. In some embodiments, the FT polypeptide according to the invention is a mature polypeptide.

In general, a FT-like variant that preserves FT-like function includes any variant in
25 which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a
30 conservative substitution as defined above.

One aspect of the invention pertains to isolated FT proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-FT antibodies. In one embodiment, native FT proteins can be isolated from cells or tissue sources by an

appropriate purification scheme using standard protein purification techniques. In another embodiment, FT proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a FT protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

5 An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the FT protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of FT protein in which the protein is
10 separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of FT protein having less than about 30% (by dry weight) of non-FT protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-FT protein, still more preferably less than about 10% of non-FT protein,
15 and most preferably less than about 5% non-FT protein. When the FT protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

20 The language "substantially free of chemical precursors or other chemicals" includes preparations of FT protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of FT protein having less than about 30% (by dry weight) of
25 chemical precursors or non-FT chemicals, more preferably less than about 20% chemical precursors or non-FT chemicals, still more preferably less than about 10% chemical precursors or non-FT chemicals, and most preferably less than about 5% chemical precursors or non-FT chemicals.

 Biologically active portions of a FT protein include peptides comprising amino
30 acid sequences sufficiently homologous to or derived from the amino acid sequence of the FT protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39 that include fewer amino acids than the full length FT proteins, and exhibit at least one activity of a FT protein, *e.g.* substrate binding. Typically, biologically active portions comprise a domain or motif with

at least one activity of the FT protein. A biologically active portion of a FT protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

A biologically active portion of a FT protein of the present invention may contain at least one of the above-identified domains conserved between the FT proteins.

5 Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native FT protein.

A biologically active portion of a FT protein can be the N-terminal domain of the FT polypeptide. Alternatively, a biologically active portion of a FT protein can be the C-terminal domain of the FT polypeptide. Preferably, the biologically active portion
10 comprises at least 75 amino acids of the C-terminal domain. More preferably, the biologically active portion comprises at least 25 amino acids of the C-terminal domain. Most preferably, the biologically active portion comprises at least 10 amino acids of the C-terminal.

15 In an embodiment, the FT protein has an amino acid sequence of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39. In other embodiments, the FT protein is substantially homologous to SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39 and retains the functional activity of the protein of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID
20 NO:33, SEQ ID NO:36 or SEQ ID NO:39, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the FT protein is a protein that comprises an amino acid sequence at least 45% homologous to the amino acid sequence of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39 and retains the functional
25 activity of the FT proteins of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:33, SEQ ID NO:36 or SEQ ID NO:39.

Determining homology between two or more sequence

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be
30 introduced in either of the sequences being compared for optimal alignment between the sequences). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used

herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. The term "percentage of positive residues" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical and conservative amino acid substitutions, as defined above, occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of positive residues.

Chimeric and fusion proteins

The invention also provides FT chimeric or fusion proteins. As used herein, a FT "chimeric protein" or "fusion protein" comprises a FT polypeptide operatively linked to a non-FT polypeptide. An "FT polypeptide" refers to a polypeptide having an amino acid sequence corresponding to FT, whereas a "non-FT polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the FT protein, *e.g.*, a protein that is different from the FT protein and that is derived from the same or a different organism. Within a FT fusion protein the FT polypeptide can correspond to all or a portion of a FT protein. In one embodiment, a FT fusion protein comprises at least one biologically active portion of a FT protein. In another embodiment, a FT fusion protein comprises at least two biologically active portions of a FT protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the FT polypeptide and the non-FT polypeptide are fused in-frame to each other. The non-FT polypeptide can be fused to the N-terminus or C-terminus of the FT polypeptide.

A FT chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide, a 6XHis-tag). A FT-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the FT protein.

FT agonists and antagonists

The present invention also pertains to variants of the FT proteins that function as either FT agonists (mimetics) or as FT antagonists. An agonist can be for example an antisense nucleic acid molecule. Variants of the FT protein can be generated by

mutagenesis, *e.g.*, discrete point mutation or truncation of the FT protein. An agonist of the FT protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the FT protein. An antagonist of the FT protein can inhibit one or more of the activities of the naturally occurring form of the FT protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the FT protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function.

Variants of the FT protein that function as either FT agonists (mimetics) or as FT antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the FT protein for FT protein agonist or antagonist activity. In one embodiment, a variegated library of FT variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of FT variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential FT sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of FT sequences therein. There are a variety of methods which can be used to produce libraries of potential FT variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential FT sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477).

Polypeptide libraries

In addition, libraries of fragments of the FT protein coding sequence can be used to generate a variegated population of FT fragments for screening and subsequent selection of variants of a FT protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a FT coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single

stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the FT protein.

5 Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of FT proteins. The most widely used techniques, which are amenable to high throughput
10 analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the
15 frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify FT variants (Arkin and Yourvan (1992) PNAS 89:7811-7815; Delgrave *et al.* (1993) Protein Engineering 6:327-331).

FT Antibodies

20 FT polypeptides, including chimeric polypeptides, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens to generate antibodies that immunospecifically-bind these peptide components. Such antibodies include, *e.g.*, polyclonal, monoclonal, chimeric, single chain, Fab fragments and a Fab expression library. In a specific embodiment, fragments of the FT polypeptides are used as
25 immunogens for antibody production. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a FT polypeptides, or derivative, fragment, analog or homolog thereof.

For the production of polyclonal antibodies, various host animals may be immunized by injection with the native peptide, or a synthetic variant thereof, or a derivative of the
30 foregoing. Various adjuvants may be used to increase the immunological response and include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.) and human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed towards a FT polypeptides, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (*see*, Kohler and
5 Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*see*, Kozbor, *et al.*, 1983. *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*see*, Cole, *et al.*, 1985. In: *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be
10 produced by the use of human hybridomas (*see*, Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see*, Cole, *et al.*, 1985. In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc., pp. 77-96).

According to the invention, techniques can be adapted for the production of
15 single-chain antibodies specific to a FT polypeptides (*see, e.g.*, U.S. Patent No. 4,946,778). In addition, methodologies can be adapted for the construction of Fab expression libraries (*see, e.g.*, Huse, *et al.*, 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a FT polypeptides or derivatives, fragments, analogs or homologs thereof. Antibody
20 fragments that contain the idiotypes to a FT polypeptides may be produced by techniques known in the art including, *e.g.*, (i) an F(ab')₂ fragment produced by pepsin digestion of an antibody molecule; (ii) an Fab fragment generated by reducing the disulfide bridges of an F(ab')₂ fragment; (iii) an Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) Fv fragments.

25 In one embodiment, methodologies for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a FT polypeptides is facilitated by generation of hybridomas that bind to the fragment of a
30 FT polypeptides possessing such a domain. Antibodies that are specific for a domain within a FT polypeptides, or derivative, fragments, analogs or homologs thereof, are also provided herein. The anti-FT polypeptide antibodies may be used in methods known within the art relating to the localization and/or quantitation of a FT polypeptide (*e.g.*, for

use in measuring levels of the peptide within appropriate physiological samples, for use in diagnostic methods, for use in imaging the peptide, and the like).

FT Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors,
5 containing a nucleic acid encoding a FT protein, or derivatives, fragments, analogs or
homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule
capable of transporting another nucleic acid to which it has been linked. One type of
vector is a "plasmid", which refers to a circular double stranded DNA loop into which
additional DNA segments can be ligated. Another type of vector is a viral vector, wherein
10 additional DNA segments can be ligated into the viral genome. Certain vectors are
capable of autonomous replication in a host cell into which they are introduced (*e.g.*,
bacterial vectors having a bacterial origin of replication). Other vectors are integrated into
the genome of a host cell upon introduction into the host cell, and thereby are replicated
along with the host genome. Moreover, certain vectors are capable of directing the
15 expression of genes to which they are operatively-linked. Such vectors are referred to
herein as "expression vectors". In general, expression vectors of utility in recombinant
DNA techniques are often in the form of plasmids. In the present specification, "plasmid"
and "vector" can be used interchangeably as the plasmid is the most commonly used form
of vector. However, the invention is intended to include such other forms of expression
20 vectors, such as viral vectors or plant transformation vectors, binary or otherwise, which
serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the
invention in a form suitable for expression of the nucleic acid in a host cell, which means
that the recombinant expression vectors include one or more regulatory sequences,
25 selected on the basis of the host cells to be used for expression, that is operatively-linked
to the nucleic acid sequence to be expressed. Within a recombinant expression vector,
"operably-linked" is intended to mean that the nucleotide sequence of interest is linked to
the regulatory sequence(s) in a manner that allows for expression of the nucleotide
sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the
30 vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and
other expression control elements (*e.g.*, polyadenylation signals). Such regulatory
sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY:
METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory

sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, FT proteins, mutant forms of FT proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of FT proteins in prokaryotic or eukaryotic cells. For example, FT proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells, plant cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein, however carboxy terminus fusions are also common. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

5 One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression
10 vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.*, Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

 In another embodiment, the FT expression vector is a yeast expression vector.
15 Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

 Alternatively, FT can be expressed in insect cells using baculovirus expression
20 vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

 In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian
25 expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both
30 prokaryotic and eukaryotic cells *see, e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

 In yet another embodiment, a nucleic acid of the invention is expressed in plants cells using a plant expression vector. Examples of plant expression vectors systems

include tumor inducing (Ti) plasmid or portion thereof found in *Agrobacterium*, cauliflower mosaic virus (CAMV) DNA and vectors such as pBI121 .

- For expression in plants, the recombinant expression cassette will contain in addition to the FT nucleic acids, a plant promoter region, a transcription initiation site (if the coding sequence to transcribed lacks one), and a transcription termination/polyadenylation sequence. The termination/polyadenylation region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. Unique restriction enzyme sites at the 5' and 3' ends of the cassette are typically included to allow for easy insertion into a pre-existing vector.
- Examples of suitable promoters include promoters from plant viruses such as the 35S promoter from cauliflower mosaic virus (CaMV). Odell, et al., *Nature*, 313: 810-812 (1985). and promoters from genes such as rice actin (McElroy, et al., *Plant Cell*, 163-171 (1990)); ubiquitin (Christensen, et al., *Plant Mol. Biol.*, 12: 619-632 (1992); and Christensen, et al., *Plant Mol. Biol.*, 18: 675-689 (1992)); pEMU (Last, et al., *Theor. Appl. Genet.*, 81: 581-588 (1991)); MAS (Velten, et al., *EMBO J.*, 3: 2723-2730 (1984)); maize H3 histone (Lepetit, et al., *Mol. Gen. Genet.*, 231: 276-285 (1992); and Atanassova, et al., *Plant Journal*, 2(3): 291-300 (1992)), the 5' or 3'-promoter derived from T-DNA of *Agrobacterium tumefaciens*, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Pat. No. 5,683,439), the Nos promoter, the rubisco promoter, the GRP1-8 promoter, ALS promoter, (WO 96/30530), a synthetic promoter, such as, Rsyn7, SCP and UCP promoters, ribulose-1,3-diphosphate carboxylase, fruit-specific promoters, heat shock promoters, seed-specific promoters and other transcription initiation regions from various plant genes, for example, include the various opine initiation regions, such as for example, octopine, mannopine, and nopaline.
- Additional regulatory elements that may be connected to a FT encoding nucleic acid sequence for expression in plant cells include terminators, polyadenylation sequences, and nucleic acid sequences encoding signal peptides that permit localization within a plant cell or secretion of the protein from the cell. Such regulatory elements and methods for adding or exchanging these elements with the regulatory elements FT gene are known, and include, but are not limited to, 3' termination and/or polyadenylation regions such as those of the *Agrobacterium tumefaciens* nopaline synthase (nos) gene (Bevan, et al., *Nucl. Acids Res.*, 12: 369-385 (1983)); the potato proteinase inhibitor II (PINII) gene (Keil, et al., *Nucl. Acids Res.*, 14: 5641-5650 (1986) and hereby incorporated by reference); and An, et

al., *Plant Cell*, 1: 115-122 (1989)); and the CaMV 19S gene (Mogen, et al., *Plant Cell*, 2: 1261-1272 (1990)).

Plant signal sequences, including, but not limited to, signal-peptide encoding DNA/RNA sequences which target proteins to the extracellular matrix of the plant cell
5 (Dratewka-Kos, et al., *J. Biol. Chem.*, 264: 4896-4900 (1989)) and the *Nicotiana plumbaginifolia* extension gene (DeLoose, et al., *Gene*, 99: 95-100 (1991)), or signal peptides which target proteins to the vacuole like the sweet potato sporamin gene (Matsuka, et al., *Proc. Nat'l Acad. Sci. (USA)*, 88: 834 (1991)) and the barley lectin gene (Wilkins, et al., *Plant Cell*, 2: 301-313 (1990)), or signals which cause proteins to be
10 secreted such as that of PR1b (Lind, et al., *Plant Mol. Biol.*, 18: 47-53 (1992)), or those which target proteins to the plastids such as that of rapeseed enoyl-ACP reductase (Verwaert, et al., *Plant Mol. Biol.*, 26: 189-202 (1994)) are useful in the invention.

In another embodiment, the recombinant expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific
15 regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Especially useful in connection with the nucleic acids of the present invention are expression systems which are operable in plants. These include systems which are under control of a tissue-specific promoter, as well as those which involve promoters that are operable in all plant tissues.

20 Organ-specific promoters are also well known. For example, the patatin class I promoter is transcriptionally activated only in the potato tuber and can be used to target gene expression in the tuber (Bevan, M., 1986, *Nucleic Acids Research* 14:4625-4636). Another potato-specific promoter is the granule-bound starch synthase (GBSS) promoter (Visser, R.G.R., et al., 1991, *Plant Molecular Biology* 17:691-699).

25 Other organ-specific promoters appropriate for a desired target organ can be isolated using known procedures. These control sequences are generally associated with genes uniquely expressed in the desired organ. In a typical higher plant, each organ has thousands of mRNAs that are absent from other organ systems (reviewed in Goldberg, P., 1986, *Trans. R. Soc. London B* 314:343).

30 For in situ production of the antisense mRNA of GST, those regions of the GST gene which are transcribed into GST mRNA, including the untranslated regions thereof, are inserted into the expression vector under control of the promoter system in a reverse orientation. The resulting transcribed mRNA is then complementary to that normally produced by the plant.

The resulting expression system or cassette is ligated into or otherwise constructed to be included in a recombinant vector which is appropriate for plant transformation. The vector may also contain a selectable marker gene by which transformed plant cells can be identified in culture. Usually, the marker gene will encode antibiotic resistance. These markers include resistance to G418, hygromycin, bleomycin, kanamycin, and gentamicin. After transforming the plant cells, those cells having the vector will be identified by their ability to grow on a medium containing the particular antibiotic. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host, preferably a broad host range prokaryotic origin of replication is included. A selectable marker for bacteria should also be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers also include resistance to antibiotics such as kanamycin or tetracycline.

Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a polypeptide of the invention encoded in an open reading frame of a polynucleotide of the invention. Accordingly, the invention further provides methods for producing a polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the polypeptide is produced. In another

embodiment, the method further comprises isolating the polypeptide from the medium or the host cell.

A number of types of cells may act as suitable host cells for expression of a polypeptide encoded by an open reading frame in a polynucleotide of the invention. Plant host cells include, for example, plant cells that could function as suitable hosts for the expression of a polynucleotide of the invention include epidermal cells, mesophyll and other ground tissues, and vascular tissues in leaves, stems, floral organs, and roots from a variety of plant species, such as *Arabidopsis thaliana*, *Nicotiana tabacum*, *Brassica napus*, *Zea mays*, and *Glycine max*.

Alternatively, it may be possible to produce a polypeptide in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous polypeptides. If the polypeptide is made in yeast or bacteria; it may be necessary to modify the polypeptide produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain a functional polypeptide; if the polypeptide is of sufficient length and conformation to have activity. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

A polypeptide may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed polypeptide or protein may then be purified from such culture (e.g., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the polypeptide or protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, a polypeptide or protein may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein containing a six-residue histidine tag. The histidine-tagged protein will then bind to a Ni-affinity column. After elution of all other proteins, the histidine-tagged protein can be eluted to

achieve rapid and efficient purification. One or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a polypeptide. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant polypeptide. The protein or polypeptide thus purified is substantially free of other plant proteins or polypeptides and is defined in accordance with the present invention as "isolated."

Transformed Plants Cells and Transgenic Plants

The invention includes protoplast, plants cells, plant tissue and plants (e.g., monocots and dicots transformed with a FT nucleic acid, a vector containing a FT nucleic acid or an expression vector containing a FT nucleic acid. Examples of nucleic acids suitable for transforming plant cells and plants include those nucleic acid sequences of SEQ ID NO: 4, 40-57 or 58. As used herein, "plant" is meant to include not only a whole plant but also a portion thereof (i.e., cells, and tissues, including for example, leaves, stems, shoots, roots, flowers, fruits and seeds).

The plant can be any plant type including, for example, species from the genera *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesis*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Pisum*, *Phaseolus*, *Lolium*, *Oryza*, *Zea*, *Avena*, *Hordeum*, *Secale*, *Triticum*, *Sorghum*, *Picea*, *Caco*, and *Populus*.

In some aspects of the invention, the transformed plant is resistant to biotic and abiotic stresses, e.g., chilling stress, salt stress, heat stress, water stress, disease, grazing pests and wound healing. Additionally, the invention also includes a transgenic plant that is resistant to pathogens such as for example fungi, bacteria, nematodes, viruses and parasitic weeds. Alternatively, the transgenic plant is resistant to herbicides. By resistant is meant the plant grows under stress conditions (e.g., high salt, decreased water, low temperatures) or under conditions that normally inhibit, to some degree, the growth of an untransformed plant. Methodologies to determine plant growth or response to stress

include for example, height measurements, weight measurements, leaf area, ability to flower, water use, transpiration rates and yield.

The invention also includes cells, tissues, including for example, leaves, stems, shoots, roots, flowers, fruits and seeds and the progeny derived from the transformed plant.

5 Numerous methods for introducing foreign genes into plants are known and can be used to insert a gene into a plant host, including biological and physical plant transformation protocols. See, for example, Miki et al., (1993) "Procedure for Introducing Foreign DNA into Plants", In: Methods in Plant Molecular Biology and Biotechnology, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pages 67-88 and Andrew Bent
10 in, Clough SJ and Bent AF, 1998. Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*.. The methods chosen vary with the host plant, and include chemical transfection methods such as calcium phosphate, polyethylene glycol (PEG) transformation, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch, et al., Science, 227: 1229-31 (1985)), electroporation, protoplast
15 transformation, micro-injection, flower dipping and particle or non-particle biolistic bombardment.

Agrobacterium-mediated Transformation

The most widely utilized method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. *A. tumefaciens* and *A.*
20 *rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectfully, carry genes responsible for genetic transformation of plants. See, for example, Kado, Crit. Rev. Plant Sci., 10: 1-32 (1991). Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided in Gruber et al., supra; and Moloney,
25 et al, Plant Cell Reports, 8: 238-242 (1989).

Transgenic *Arabidopsis* plants can be produced easily by the method of dipping flowering plants into an *Agrobacterium* culture, based on the method of Andrew Bent in, Clough SJ and Bent AF, 1998. Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Wild type plants are grown until the
30 plant has both developing flowers and open flowers. The plant are inverted for 1 minutes into a solution of *Agrobacterium* culture carrying the appropriate gene construct. Plants are then left horizontal in a tray and kept covered for two days to maintain humidity and then righted and bagged to continue growth and seed development. Mature seed was bulk harvested.

Direct Gene Transfer

A generally applicable method of plant transformation is microprojectile-mediated transformation, where DNA is carried on the surface of microprojectiles measuring about 1 to 4 μm . The expression vector is introduced into plant tissues with a biolistic device
5 that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate the plant cell walls and membranes. (Sanford, et al., Part. Sci. Technol., 5: 27-37 (1987); Sanford, Trends Biotech, 6: 299-302 (1988); Sanford, Physiol. Plant, 79: 206-209 (1990); Klein, et al., Biotechnology, 10: 286-291 (1992)).

Another method for physical delivery of DNA to plants is sonication of target cells
10 as described in Zang, et al., BioTechnology, 9: 996-996 (1991). Alternatively, liposome or spheroplast fusions have been used to introduce expression vectors into plants. See, for example, Deshayes, et al., EMBO J., 4: 2731-2737 (1985); and Christou, et al., Proc. Nat'l. Acad. Sci. (USA), 84: 3962-3966 (1987). Direct uptake of DNA into protoplasts using CaCl_2 precipitation, polyvinyl alcohol or poly-L-ornithine have also been reported. See,
15 for example, Hain, et al., Mol. Gen. Genet., 199: 161 (1985); and Draper, et al., Plant Cell Physiol., 23: 451-458 (1982).

Electroporation of protoplasts and whole cells and tissues has also been described. See, for example, Donn, et al., (1990) In: Abstracts of the VIIth Intl. Congress on Plant Cell and Tissue Culture IAPTC, A2-38, page 53; D'Halluin et al., Plant Cell, 4: 1495-1505
20 (1992); and Spencer et al., Plant Mol. Biol., 24: 51-61 (1994).

Plants may also be transformed using the method of Held et al. (U.S. Application 20010026941). The method utilizes an accelerated aerosol beam of droplets which carries the desired molecules, DNA, into the target cells. The size of droplets produced by this method are reported to be sufficiently small as to transform bacterial cells of 1 to 2
25 microns in length.

Particle Wounding/Agrobacterium Delivery

Another useful basic transformation protocol involves a combination of wounding by particle bombardment, followed by use of *Agrobacterium* for DNA delivery, as
30 described by Bidney, et al., Plant Mol. Biol., 18: 301-31 (1992). Useful plasmids for plant transformation include Bin 19. See Bevan, Nucleic Acids Research, 12: 8711-8721 (1984), and hereby incorporated by reference.

In general, the intact meristem transformation method involves imbibing seed for 24 hours in the dark, removing the cotyledons and root radical, followed by culturing of

the meristem explants. Twenty-four hours later, the primary leaves are removed to expose the apical meristem. The explants are placed apical dome side up and bombarded, e.g., twice with particles, followed by co-cultivation with *Agrobacterium*. To start the co-cultivation for intact meristems, *Agrobacterium* is placed on the meristem. After about a
5 3-day co-cultivation period the meristems are transferred to culture medium with cefotaxime plus kanamycin for the NPTII selection.

The split meristem method involves imbibing seed, breaking of the cotyledons to produce a clean fracture at the plane of the embryonic axis, excising the root tip and then bisecting the explants longitudinally between the primordial leaves. The two halves are
10 placed cut surface up on the medium then bombarded twice with particles, followed by co-cultivation with *Agrobacterium*. For split meristems, after bombardment, the meristems are placed in an *Agrobacterium* suspension for 30 minutes. They are then removed from the suspension onto solid culture medium for three day co-cultivation. After this period, the meristems are transferred to fresh medium with cefotaxime plus kanamycin for
15 selection.

Transfer by Plant Breeding

Alternatively, once a single transformed plant has been obtained by the foregoing recombinant DNA method, conventional plant breeding methods can be used to transfer the gene and associated regulatory sequences via crossing and backcrossing. Such
20 intermediate methods will comprise the further steps of: (1) sexually crossing the disease-resistant plant with a plant from the disease susceptible taxon; (2) recovering reproductive material from the progeny of the cross; and (3) growing disease-resistant plants from the reproductive material. Where desirable or necessary, the agronomic characteristics of the susceptible taxon can be substantially preserved by expanding this method to include the
25 further steps of repetitively: (1) backcrossing the disease-resistant progeny with disease-susceptible plants from the susceptible taxon; and (2) selecting for expression of a hydrogen peroxide producing enzyme activity (or an associated marker gene) among the progeny of the backcross, until the desired percentage of the characteristics of the susceptible taxon are present in the progeny along with the gene or genes imparting oxalic
30 acid degrading and/or hydrogen peroxide enzyme activity.

By the term "taxon" herein is meant a unit of botanical classification. It thus includes, genus, species, cultivars, varieties, variants and other minor taxonomic groups which lack a consistent nomenclature.

Regeneration of Transformants

The development or regeneration of plants from either single plant protoplasts or various explants is well known in the art (Weissbach and Weissbach, 1988). This regeneration and growth process typically includes the steps of selection of transformed
5 cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene
10 that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described (Horsch et al., 1985). In this procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant strain being transformed as described (Fraley et al., 1983). In particular, U.S. Pat. No. 5,349,124 (specification
15 incorporated herein by reference) details the creation of genetically transformed lettuce cells and plants resulting therefrom which express hybrid crystal proteins conferring insecticidal activity against Lepidopteran larvae to such plants.

This procedure typically produces shoots within two to four months and those shoots are then transferred to an appropriate root-inducing medium containing the
20 selective agent and an antibiotic to prevent bacterial growth. Shoots that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain employed, such variations being well known in the art.

Preferably, the regenerated plants are self-pollinated to provide homozygous
25 transgenic plants, or pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important, preferably inbred lines. Conversely, pollen from plants of those important lines is used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

30 A preferred transgenic plant is an independent segregant and can transmit the FT gene and its activity to its progeny. A more preferred transgenic plant is homozygous for the gene, and transmits that gene to all of its offspring on sexual mating. Seed from a transgenic plant may be grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from

these plants become true breeding lines that are evaluated for increased expression of the FT transgene.

Method of Producing Transgenic Plants

Included in the invention are methods of producing a transgenic plant that has increased stress resistance, delayed senescence or increased sensitivity to ABA. The method includes introducing into one or more plant cells a compound that alters farnesyl transferase expression (i.e. farnesyl transferase alpha or beta) or activity in the plant. The compound can be, *e.g.*, (i) a farnesyl transferase polypeptide inhibitor; (ii) a nucleic acid encoding a farnesyl transferase polypeptide inhibitor; (iii) a nucleic acid that decreases expression of a nucleic acid that encodes a farnesyl transferase polypeptide and, derivatives, fragments, analogs and homologs thereof; (iv) an antisense farnesyl transferase nucleic acid. A nucleic acid that decreases expression of a nucleic acid that encodes a farnesyl transferase polypeptide includes, *e.g.*, antisense nucleic acids or RNA inhibitory nucleic acids. The nucleic acid can be either endogenous or exogenous. Preferably the compound is a farnesyl transferase polypeptide or a nucleic acid encoding a farnesyl transferase polypeptide. For example the compound is the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37. More preferably the compound is a nucleic acid complementary to a nucleic acid encoding a farnesyl transferase polypeptide. For example an anti-sense nucleic acid molecule. Exemplary compounds include SEQ ID NO: 1, 3, 4, 29, 30, 32, 35, 38, 40 -57 and 58.

Also included in the invention is a plant where a mutation has been introduced in the gene encoding farnesyl transferase (i.e. alpha or beta) which results in a plant that has decreased farnesyl transferase activity and increased tolerance to stress as compared to a wild type plant. The mutation may be introduced by chemical or mechanical means.

Examples of stresses include, for example, chilling stress, heat stress, salt stress, water stress, nutrient limitation stress, disease, grazing pests, wound healing, pathogens such as for example fungi, bacteria, nematodes, viruses or parasitic weed and herbicides.

Increased stress resistance is meant that the transgenic plant can grow under stress conditions (*e.g.*, high salt, decreased water, low temperatures) or under conditions that normally inhibit the growth of an untransformed plant. Methodologies to determine plant growth or response to stress include for example, height measurements, weight measurements, leaf area, ability to flower, water use, transpiration rates and yield

Sensitivity to ABA can be assessed using a concentration curve of ABA and germinating seeds on plates as described in Example 11. Often germination is assessed and used to determine sensitivity. However, sensitivity can be observed at more developmental stages than simply germination. For example, increased sensitivity may be observed at the stage of cotyledon expansion, expansion of the first true leaf, or developmental arrest in the seedling stage.

The concentration of ABA at which sensitivity is observed varies in a species dependent manner. For example, transgenic *Arabidopsis thaliana* will demonstrate sensitivity at a lower concentration than observed in *Brassica* or soybean.

By increased ABA sensitivity it is meant that the transgenic plant is seen to display a phenotype at a lower concentration of ABA than that used to observe the same phenotype in a wild type plant. Methodologies to determine ABA sensitivity include for example, plant germination, growth or development .

The plant can be any plant type including, for example, species from the genera *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesis*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browallia*, *Glycine*, *Pisum*, *Phaseolus*, *Lolium*, *Oryza*, *Zea*, *Avena*, *Hordeum*, *Secale*, *Triticum*, *Sorghum*, *Picea*, *Caca*, and *Populus*.

Screening Methods

The isolated nucleic acid molecules of the invention (e.g., SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:31, SEQ ID NO:34, or SEQ ID NO:37) can be used to express FT protein (e.g., via a recombinant expression vector in a host cell), to detect FT mRNA (e.g., in a biological sample) or a genetic lesion in a FT gene, and to modulate FT activity, as described further, below. In addition, the FT proteins can be used to screen compounds that modulate the FT protein activity or expression. In addition, the anti-FT antibodies of the invention can be used to detect and isolate FT proteins and modulate FT activity.

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to FT proteins or have a stimulatory or inhibitory effect on, *e.g.*, FT protein expression or FT protein activity. The invention also includes compounds identified in the screening assays described herein. The invention also includes methods of identifying related genes using the transgenic plants of this invention in screening protocols utilizing mutagenesis, gene tagging, insertional gene tagging, activation tagging or other such methods of gene or phenotype identification.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to a FT protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores

(Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

5 In one embodiment, an assay is a cell-based assay in which a cell which expresses a FT protein, or a biologically-active portion thereof, is contacted with a test compound and the ability of the test compound to bind to a FT protein determined. The cell, for example, can be of mammalian origin, plant cell or a yeast cell. Determining the ability of the test compound to bind to the FT protein can be accomplished, for example, by

10 coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the FT protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test

15 compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a FT protein, or a biologically-active portion thereof, with a known compound which binds FT to form an assay mixture, contacting the

20 assay mixture with a test compound, and determining the ability of the test compound to interact with a FT protein, wherein determining the ability of the test compound to interact with a FT protein comprises determining the ability of the test compound to preferentially bind to FT protein or a biologically-active portion thereof as compared to the known compound.

25 In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a FT protein, or a biologically-active portion thereof, with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FT protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of FT or a biologically-active portion thereof

30 can be accomplished, for example, by determining the ability of the FT protein to bind to or interact with a FT target molecule. As used herein, a "target molecule" is a molecule with which a FT protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a FT interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal

surface of a cell membrane or a cytoplasmic molecule. A FT target molecule can be a non-FT molecule or a FT protein or polypeptide of the invention. In one embodiment, a FT target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with FT.

Determining the ability of the FT protein to bind to or interact with a FT target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the FT protein to bind to or interact with a FT target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+} , diacylglycerol, IP_3 , etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a FT-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.* luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting a FT protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the FT protein or biologically-active portion thereof. Binding of the test compound to the FT protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the FT protein or biologically-active portion thereof with a known compound which binds FT to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FT protein, wherein determining the ability of the test compound to interact with a FT protein comprises determining the ability of the test compound to preferentially bind to FT or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting FT protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the FT protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of FT can be accomplished, for example, by

determining the ability of the FT protein to bind to a FT target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of FT protein can be accomplished by determining the ability of the FT protein further modulate a FT target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described above.

In yet another embodiment, the cell-free assay comprises contacting the FT protein or biologically-active portion thereof with a known compound which binds FT protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FT protein, wherein determining the ability of the test compound to interact with a FT protein comprises determining the ability of the FT protein to preferentially bind to or modulate the activity of a FT target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of FT protein. In the case of cell-free assays comprising the membrane-bound form of FT protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of FT protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either FT protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to FT protein, or interaction of FT protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-FT fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then

combined with the test compound or the test compound and either the non-adsorbed target protein or FT protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix
5 immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of FT protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the
10 screening assays of the invention. For example, either the FT protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated FT protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce
15 Chemical). Alternatively, antibodies reactive with FT protein or target molecules, but which do not interfere with binding of the FT protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or FT protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of
20 complexes using antibodies reactive with the FT protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FT protein or target molecule.

In another embodiment, modulators of FT protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of FT
25 mRNA or protein in the cell is determined. The level of expression of FT mRNA or protein in the presence of the candidate compound is compared to the level of expression of FT mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of FT mRNA or protein expression based upon this comparison. For example, when expression of FT mRNA or protein is greater
30 (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of FT mRNA or protein expression. Alternatively, when expression of FT mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of FT mRNA or protein expression. The

level of FT mRNA or protein expression in the cells can be determined by methods described herein for detecting FT mRNA or protein.

In yet another aspect of the invention, the FT proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with FT ("FT-binding proteins" or "FT-bp") and modulate FT activity. Such FT-binding proteins are also likely to be involved in the propagation of signals by the FT proteins as, for example, upstream or downstream elements of the FT pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for FT is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a FT-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with FT.

In yet another aspect of the invention are methods which utilize the transgenic plants of the invention to identify FT-interacting components via genetic screening protocols. These components can be for example, regulatory elements which modify FT-gene expression, interacting proteins which directly modify FT activity or interacting proteins which modify components of the same signal transduction pathway and thereby exert an effect on the expression or activity of FT. Briefly, genetic screening protocols are applied to the transgenic plants of the invention and in so doing identify related genes which are not identified using a wild type background for the screen. For example an activation tagged library (Weigel, *et al.*, 2000. *Plant Physiol.* 122: 1003-1013), can be produced using the transgenic plants of the invention as the genetic background. Plants are

then screened for altered phenotypes from that displayed by the parent plants. Alternative methods of generating libraries from the transgenic plants of the invention can be used, for example, chemical or irradiation induced mutations, insertional inactivation or activation methods.

5 The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof.

 The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing
10 description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

 The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

15

EXAMPLES

Example 1: Cloning of *Arabidopsis thaliana* FTA and Construction of Transformation Vector

 The *Arabidopsis thaliana* FTA sequence was obtained by RT-PCR from total RNA isolated from leaf tissue using primers corresponding to SEQ ID NO:11 and SEQ ID
20 NO:12. The resulting fragment was digested with *Bam*HI and *Sma*I and cloned into the plasmid pCR2.1 The Clontech vector pBI121 was used as the backbone for the antisense construct. The GUS gene was removed by *Bam*HI and *Eco*1CRI digestion and replaced with the FTA insert that was cut from pCR2.1-FTA using *Sma*I and *Bam*HI and ligated into the vector SEQ ID NO:4.

25 **Table 1.**

SEQ ID NO:11: 5' – AAAGGATCCTCAAATTGCTGCCACTGTAAT –3'

SEQ ID NO:12: 5' – AAACCCGGGATGAATTTTCGACGAGAACGTG –3'

30 **Example 2: Cloning of non-full length *Brassica napus* FTA and FTB nucleic acid sequences**

 RNA was isolated from leaf and root tissue using the Qiagen RNeasy kit. RT-PCR was performed by known techniques using the primers shown in Table 2. The FTA

sequence was obtained using the primer pair SEQ ID NO:19 and SEQ ID NO:20. The FTB sequence was obtained using the primer pair SEQ ID NO:21 and SEQ ID NO:22.

Table 2.

5	SEQ ID NO:19:	5' -GGATCCATGGATTACTTCCGTGCGATTTACTTCTCC-3'
	SEQ ID NO:20:	5' -AAAAAGCTTCCATGCCCAATAGTTAGCTCTTATTGGATC-3'
	SEQ ID NO:21:	5' -AAAAAGCTTTGGCTTTGTTACTGGATTCTTCATTCAAT-3'
	SEQ ID NO:22:	5' -AAATCTAGAAGCTTCATAATACCGATCCAAGACAATGTT-3'

10 PCR products were separated from the RT-PCR reaction mixture using the Qiagen PCR column spin kit and ligated into the cloning vector pBluescript KS+. The vector was digested with *EcoRV* and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhang for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5 α cells, positive colonies were selected and the resulting inserts
15 sequenced.

Example 3: Cloning of non-full length FTA and FTB nucleic acid sequences from *Glycine max* and *Zea mays*

RNA was isolated from leaf and root tissue using the Qiagen RNeasy kit. RT-PCR was performed by known techniques using the primers shown in Table 3. The *Glycine max*
20 FTA sequence was obtained using the primer pair SEQ ID NO:23 and SEQ ID NO:24. The *Glycine max* FTB sequence was obtained using the primer pair SEQ ID NO:25 and SEQ ID NO:26. The *Zea mays* FTB sequence was obtained using the primer pair SEQ ID NO:27 and SEQ ID NO:28.

25 **Table 3.**

	SEQ ID NO:23:	5' -AAAGGATCCATGGAATCTGGGTCTAGCGA-3'
	SEQ ID NO:24:	5' -AAATCTAGAAGGAAGTCTGCTCTTGCGC-3'
	SEQ ID NO:25:	5' -AAATCTAGAGCCACCATTCTCGCAACG-3'
30	SEQ ID NO:26:	5' -AAAGAGCTCGTGGTGGAGAATCTGGGTGC-3'
	SEQ ID NO:27:	5' -GGCGGATCCCGACCTACCGAGG-3'
	SEQ ID NO:28:	5' -AAAGAGCTCGTGGATGGATTGGCTCCAGC-3'

PCR products were separated from the RT-PCR reaction mixture using the Qiagen
35 PCR column spin kit and ligated into the cloning vector pBluescript KS+. The vector was

digested with *EcoRV* and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhang for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5 α cells, positive colonies were selected and the resulting inserts sequenced.

5 Example 4: Sequence Analysis

Arabidopsis thaliana FTA

A disclosed nucleic acid of 999 nucleotides (also referred to as FT1) is shown in Table 4A. The primers used in the PCR are depicted in bold.

Table 4A. FT1 Nucleotide Sequence (SEQ ID NO:1).

aaaccgaggatgaatttcgacgagaccgtgccactgagccaacgattggagtggcagacgtgggt
cccattgactcaggacgatgggtccgaatccagtgggtgccaattgcctacaaggaagagttccgcg
agactatggattacttccgtgcgatttacttttccgacgagcgatctcctcgcgccactacgactc
acggaagaaacctcctcttaaactccggcaactacacagtgtggcatttcaggcgccctagtact
cgaggcccttaatacagacttgtttgaagaactcgagttcatcgaaacgcattgctgaggataact
ctaagaactaccaactgtggcatcatcggcgatgggttgagagaaactgggtcctgatgttgca
gggagagaacttgaatttaccgtagagtactttcacttgatgccaacattatcatgcttgggtc
acataggcagtggaactacgggcattaggaggatgggaagatgagctcgattactgtcacgagc
tccttgaagctgacgtctttaacaattccgcttgaatcagaggtattatgtcatcacccaatct
cctttgttgggaggcctagaagccatgagagaatctgaagtaagctacacaatcaaagccatttt
aaccaatcctgcaaacgagagctcatggcgatacctaaaagcgctttacaaagacgacaaagaat
cctggattagtgatccaagtgtttcctcagtcgtttgaatgttctatcccgcacagattgcttc
catggattcgctctgagcacccttttgatcttctatgtgatggactgagaccaaccaacgagca
taaagactcagtgagagctctagctaataagaacacagagactaacttgccaatttggtgtgta
ctattccttggtcgtagatcctataagagctaactattgggcgatggaggaagagcaagatt**aca**
gtggcagcaatttgaggatccttt

10

A disclosed FT1 polypeptide (SEQ ID NO:5) encoded by SEQ ID NO:1 has 326 amino acid residues and is presented in Table 4B using the one-letter amino acid code.

Table 4B. Encoded FT1 protein sequence (SEQ ID NO:5).

MNFDETVPLSQRLWSDVPLTQDDGPNPVVPIAYKEEFRETMDYFRAIFYSDERSPRALRLTE
ETLLNSGNYTVWHFRRLVLEALNHDLFEELEFIERIAEDNSKNYQLWHHRRWVAEKLGPDVAG
RELEFTRRVLSLDAKHYPHAWSHRQWTLRALGGWEDEL DYCHELLEADVFNNSAWNQRYYVITQS
PLGGLEAMRESEVSYTIKAILTNPANESSWRYLKALYKDDKESWISDPSVSSVCLNVLSRTDC
FHGFALSTLLDLLCDGLRPTNEHKDSVRALANEEPETNLANLVCTILGRVDPIRANYWAWRKSK
ITVAAI

Due to the nature of the cloning strategy the sequence presented does not contain
any 5' or 3' non-translated sequence. Using the sequences disclosed herein as hybridization
probes, one is able to screen and isolate full length sequences from cDNA or genomic
libraries or use the rapid amplification of cDNA ends (RACE) technology or other such
PCR techniques. The percent identity of the *Arabidopsis thaliana* nucleotide sequence and
its encoded amino acid sequence to that of published sequences is shown in Figure 8.

The present invention also includes a nucleic acid sequence complimentary to the *Arabidopsis thaliana* farnesyl transferase alpha subunit of SEQ ID NO:1. The disclosed complementary sequence is shown as SEQ ID NO:2. The nucleic acid sequence of SEQ ID NO:3 shows the nucleic acid sequence of SEQ ID NO:2 that has been prepared for ligation

5 into an expression vector.

SEQ ID NO:2

aaaggatcctcaaattgctgccactgtaatccttgctcttccctccatgcccaatagtttagctcttataggatc
 10 tacacgaccaagaatagtagacacaccaaattggccaagtttagtctctggttcttcatttagctagagctctcac
 tgagtctttatgctcgttgggttggtctcagtcctcatcacatagaagatccaaaaggtgctcagagcgaatcc
 atggaagcaatctgtgcgggatagaacattcaaacagactgaggaaacacttggatcactaatccaggattc
 tttgtcgtctttgtaaagcgcttttaggtatcgccatgagctctcggttgcaggattggttaaaatggcttt
 gattgtgtagcttacttcagattctctcatggcttctaggcctcccaacaaaggagattgggtgatgacata
 atacctctgattccaggcggaattgttaaagacgtcagcttcaaggagctcgtgacagtaatcgagctcatc
 15 ttcccatcctcctaattgcccgtagtgtccactgcctatgtgaccaagcatgataatgtttggcatcaagtga
 aagtactctacgggtaaattcaagttctctccctgcaacatcaggacccagtttctctgcaacccatcgccg
 atgatgccacagttggtagttcttagagttatcctcagcaatgcgttcgatgaactcgagttcttcaaaciaa
 gtcgtgattaagggcctcgagtactaggcgctgaaatgccacactgtgtagttgccggagtttaagaggag
 ggtttcttccgtgagtcgtagtgcgcgaggagatcgctcgtcggaagtaaatcgacggaagtaatccat
 20 agtctcgcggaactcttccctgtaggcaattggcaccactggattcggaaccatcgctcctgagtcaatgggac
 cagctctgaccactccaatcgttgggtcagtgga**cagggtctcgctcgaaattcatccccgggttt**

SEQ ID NO:3

gatcctcaaattgctgccactgtaatccttgctcttccctccatgcccaatagtttagctcttataggatctaca
 25 cgaccaagaatagtagacacaccaaattggccaagtttagtctctggttcttcatttagctagagctctcactgag
 tctttatgctcgttgggttggtctcagtcctcatcacatagaagatccaaaaggtgctcagagcgaatccatgg
 aagcaatctgtgcgggatagaacattcaaacagactgaggaaacacttggatcactaatccaggattctttg
 tcgtctttgtaaagcgcttttaggtatcgccatgagctctcggttgcaggattgggttaaaatggctttgatt
 gtgtagcttacttcagattctctcatggcttctaggcctcccaacaaaggagattgggtgatgacataatac
 30 ctctgattccaggcggaattgttaaagacgtcagcttcaaggagctcgtgacagtaatcgagctcatcttcc
 catcctcctaattgcccgtagtgtccactgcctatgtgaccaagcatgataatgtttggcatcaagtgaaggt
 actctacgggtaaattcaagttctctccctgcaacatcaggacccagtttctctgcaacccatcgccgatga
 tgccacagttggtagttcttagagttatcctcagcaatgcgttcgatgaactcgagttcttcaaacaagtcg
 tgattaagggcctcgagtactaggcgctgaaatgccacactgtgtagttgccggagtttaagaggagggtt
 35 tcttccgtgagtcgtagtgcgcgaggagatcgctcgtcggaagtaaatcgacggaagtaatccatagtc
 tcgcggaactcttccctgtaggcaattggcaccactggattcggaaccatcgctcctgagtcaatgggaccacg
 tctgaccactccaatcgttgggtcagtgga**cagggtctcgctcgaaattcatccc**

Brassica napus FTA

40 A disclosed nucleic acid of 822 nucleotides (also referred to as FT2) is shown in Table 5A.

Table 5A. FT2 Nucleotide Sequence (SEQ ID NO:6).

ATGGATTACTTCCGTGCGATTACTTCTCCGACGAGCGTTCTGCTCGCGCGCTGCGACTCACGGA
 AGAAGCTCTCCGCTTAACTCGGGCAACTACACCGTGTGGCACTTCGGGCGCTTAGTACTCGAGG
 AGCTTAATAACGACTTGTATGAAGAGCTCAAGTTCATCGAAAGCATTGCTGAGGATAACTCTAAG
 AACTACCAGTTGTGGCATCATCGACGATGGGTCGAGAGAACTGGGTCCTGATGTTGCAGGAAA
 GGAAGTTGAGTTTACTCGGAGGGTACTATCACTTGATGCCAAGCATTATCATGCTTGGTCACATA
 GGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAAATGAGCTTAACTACTGCCACGAGCTCCTT
 GAAGCTGACGTCTTTAACAACCTCTGCATGGAATCAGAGGTATTACGTTATAACTAGATCACCTTC
 GTTGGGAGGCCTAGAAGCCATGAGAGAATCTGAAGTAAGCTACACAGTCAAAGCCATTTTAGCAA
 ATCCCGGGAACGAGAGCTCTTGGAGGTACCTGAAAGCCCTTTACAAAGACGACACAGAGTCTTGG
 ATTAGTGATCCAAGTGTTTCTCAGTCTGTTTGAAAGTTCTCTCACGCGCGGACTGCTTCCATGG
 ATTCGCTCTGAGCACCTTTTGGATCTTCTGTGCGATGGGTTGAGACCAACCAACGAGCATAGAG
 ACTCGGTGAAAGCTCTAGCTAATGAAGAACCAGAGACTAACTTGCCAATTTGGTGTGTACCATT
 CTGTGTCGTGTTGATCCAATAAGAGCTAACTATTGGGCATGG

A disclosed FT2 polypeptide (SEQ ID NO:7) encoded by SEQ ID NO:6 has 274 amino acid residues and is presented in Table 5B using the one-letter amino acid code.

Table 5B. Encoded FT2 protein sequence (SEQ ID NO:7).

MDYFRAIYFSDERSARALRLTEEALRLNSGNYTVWHFGRVLVEELNNDLYEELKFIESIAEDNS
 KNYQLWHHRRWVAEKLGPDVAGLEKEFTRRVLSDAKHYHAWSHRQWALQALGGWENELNYCHE
 LLEADVFNNSAWNQRYYVITRSPSLGGLEAMRESEVSYTVKAILANPGNESSWRYLKALYKDDT
 ESWISDPVSSVCLKVLSRADCFHGFALSTLLDLLCDGLRPTNEHRDSVKALANEEPETNLNL
 VCTILCRVDPIRANYWAWKL

5 Due to the nature of the cloning strategy the sequence presented is not full length. Compared to the *Arabidopsis thaliana* sequence there are 42 amino acids missing from the amino terminus and 10 amino acids from the carboxy terminus. The percent identity of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 8.

10 Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napus* farnesyl transferase alpha subunit of SEQ ID NO:6. The disclosed
 15 complimentary sequence is shown as SEQ ID NO:29.

SEQ ID NO:29

CCATGCCCAATAGTTAGCTCTTATTGGATCAACACGACACAGAATGGTACACACCAAATTGGCCAAGTTAGT
 CTCTGGTTCTTCATTAGCTAGAGCTTTCACCGAGTCTCTATGCTCGTTGGTTGGTCTCAACCCATCGCACAG
 20 AAGATCCAAAAGGGTGCTCAGAGCGAATCCATGGAAGCAGTCCGCGCGTGAGAGAACTTCAAACAGACTGA
 GGAAACACTTGGATCACTAATCCAAGACTCTGTGTCGTCTTTGTAAAGGGCTTTCAGGTACCTCCAAGAGCT
 CTCGTTCCCGGGATTTGCTAAAATGGCTTTGACTGTGTAGCTTACTTCAGATTCTCTCATGGCTTCTAGGCC
 TCCCAACGAAGGTGATCTAGTTATAACGTAATACCTCTGATTCCATGCAGAGTTGTTAAAGACGTCAGCTTC
 AAGGAGCTCGTGGCAGTAGTTAAGCTCATTTTCCCATCCTCCTAATGCTTGTAGCGCCCACTGCCTATGTGA
 25 CCAAGCATGATAATGCTTGGCATCAAGTGATAGTAGTACCCTCCGAGTAAACTCAAGTTCCTTCCCTGCAACATC
 AGGACCCAGTTTCTCTGCGACCCATCGTCGATGATGCCACAAGTGGTAGTTCTTAGAGTTATCCTCAGCAAT
 GCTTTCGATGAACTTGAGCTCTTCATACAAGTCGTTATTAAGCTCCTCGAGTACTAAGCGCCCGAAGTGCCA

CACGGTGTAGTTGCCCCGAGTTTAAGCGGAGAGCTTCTTCCGTGAGTCGCAGCGCGGAGCAGAACGCTCGTC
GGAGAAGTAAATCGCACGGAAGTAATCCAT

Brassica napus FTB

- 5 A disclosed nucleic acid of 1110 nucleotides (also referred to as FT3) is shown in Table 6A.

Table 6A. FT3 Nucleotide Sequence (SEQ ID NO:8).
TGGCTTTGTTACTGGATTCTTCATTCAATTGCTTTGCTTGGGGAGTCTGTGGATGATGACTTAGA AAACAATGCAATCGATTTTCTTGGACGTTGCCAGGGTTCTGATGGTGGATATGGTGGTGGTCCTG GCCAACTTCCACATCTTGCAACAAGTTATGCTGCAGTGAATACACTTGTTACTTTAGGAGGTGAG AAAGCCTTCTCTTCAATTAACAGAGAACAATGGCTTGTTTCTTAAGACGAATGAAGGATACAAA TGGAGGTTTCAGGATGCATAATATGGGAGAAATAGATGTGCGAGCGTGCTACACTGCGATTTTGA TTGCAAGCATCCTGAACATTGTGGATGATGAACTCACCCGCGGCTTAGGAGATTACATTTTGAGT TGCCAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGAAGCTCATGGTGGGTACACGTA CTGTGGGTGGCTACTATGATTTTAATCAATGAAGTCGACCGCTTGAATTTGGATTTCGTTAATGA ATTGGGTGTACATCGACAAGGAGTAGAAATGGGATTCCAAGGTAGGACGAACAAATTGGTCGAC GGTGCTACACGTTTTGGCAGGCAGCCCCCTGTGTTCTACTACAGCGATTTTTTTCATCCCAGGA TATGGCACCTCATGGATCATCATCACATATGTCACAAGGGACAGATGAAGATCACGAGGAACATG GTCATGATGAAGATGATCCTGAAGACAGTGATGAAGATGATTCTGATGAGGATAGCGATGAAGAT TCAGGGAATGGTCACCAAGTTCATCATACGTCTACCTACATTGACAGGAGAATTCAACCTGTTTT TGATAGCCTCGGCTTGCAAAGATATGTGCTCTTGCTCTCAGGTTGCTGATGGTGGATTCAGAG ACAAGCTGAGGAACCCCGTGACTTCTACCACACATGTTACTGCCTAAGCGGTCTTTCCGTGGCT CAACACGCTTGGTCAAAGACGAGGACACTCCTCCTTTGACTCGTGACATTTTGGGTGGCTACGC AAACCACCTTGAACCTGTTACCTCCTCCACAACATTGTCTTGGATCGGTATTATGAAGCTTCTA GATT

A disclosed FT3 polypeptide (SEQ ID NO:9) encoded by SEQ ID NO:7 has 370 amino acid residues and is presented in Table 6B using the one-letter amino acid code.

Table 6B. Encoded FT3 protein sequence (SEQ ID NO:9).
WLCYWILHSIALLGESVDDDLNNAIDFLGRCQGSDDGGYGGGPGQLPHLATSYYAAVNTLVTLGG EKAFSSINREQMACFLRRMKDTNGGFRMHNMG EIDVRACYTAILIASILNIVDELTRGLGDYI LSCQTYEGGIGGEPGSEAHGGYTYCGLATMILINEVDRLNLDLSLMNWVVRQGVEMGFQGRNKG LVDGCTFWQAAPCVLLQRFSSQDMPHGGSSSHMSQGTDEDHEEHGHDEDDPEDSDEDDSDDED SDEDSGNHGHVHHTSTYIDRRIQPVFDSLGLQRYVLLCSQVADGGFRDKLRKPRDFYHTCYCLS GLSVAQHAWSKDEDTPLTRDILGGYANHLEPVHLLHNILVDRYYEASRF

10

- Due to the nature of the cloning strategy the sequence presented is not full length. Compared to the *Arabidopsis thaliana* sequence there are 31 amino acids missing from the amino terminus and 5 amino acids from the carboxy terminus. The percent identity of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 9.
- 15

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques. Sequence

comparisons have been performed and percent identities are shown in Figure 8 and Figure 9.

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napsus* farnesyl transferase beta subunit of SEQ ID NO:8. The disclosed
5 complimenary sequence is shown as SEQ ID NO:30.

SEQ ID NO:30

AAATCTAGAAGCTTCATAATACCGATCCAAGACAATGTTGTGGAGGAGGTGAACAGGTTCAAGGTGGTTTGC
GTAGCCACCCAAAATGTCACGAGTCAAAGGAGGAGTGTCTCTGTTTGGACCAAGCGTGTGAGCCACGGA
10 AAGACCGCTTAGGCAGTAACATGTGTGGTAGAAGTCACGGGGTTTCTCAGCTTGTCTCTGAATCCACCATC
AGCAACCTGAGAGCACAAGAGCACATATCTTTGCAAGCCGAGGCTATCAAAAACAGGTTGAATTCTCCTGTC
AATGTAGGTAGACGTATGATGAACTTGGTGACCATTCCTGAATCTTCATCGCTATCCTCATCAGAATCATC
TTCATCACTGTCTTCAGGATCATCTTCATCATGACCATGTTCTCTCGTGATCTTCATCTGTCCCTTGTGACAT
ATGTGATGATGATCCATGAGGTGCCATATCTTGGGATGAAAAAATCGCTGTAGTAGAACACAGGGGGCTGC
15 CTGCCAAAACGTGTAGCAACCGTCGACCAATTTGTTTCGTCTACCTTGAATCCCATTCTACTCCTTGTCTG
ATGTACAACCCAATTCATTAACGAATCCAAATTCAGCGGTGCACTTCATTGATTAATAATCATAGTAGCCAA
CCCACAGTACGTGTACCCACCATGAGCTTCGGAGCCAGGTTCCCTCCAATGCCACCTTCATAAGTTTGGCA
ACTCAAAATGTAATCTCCTAAGCCGCGGGTGAGTTCATCATCCACAATGTTTCAAGATGCTTGCAATCAAAAT
CGCAGTGTAGCAGCTCGCACATCTATTTCTCCCATATTATGCATCCTGAAACCTCCATTTGTATCCTTCAT
20 TCGTCTTAAGAAACAAGCCATTTGTTCTCTGTTAATTGAAGAGAAGGCTTTCTCACCTCCTAAAGTAACAAG
TGATTCTACTGCAGCATAAATTGTTGCAAGATGTGGAAGTTGGCCAGGACCACCACCATATCCACCATCAGA
ACCTTGGCAACGTCCAAGAAAATCGATTGCATGTTTTCTAAGTCATCATCCACAGACTCCCCAAGCAAAGC
AATTGAATGAAGAATCCAGTAACAAAGCCA

Glycine max FTA

25 A disclosed nucleic acid of 1041 nucleotides (also referred to as FT4) is shown in Table 7A.

Table 7A. FT4 Nucleotide Sequence (SEQ ID NO:31).

ATGGAATCTGGGTCTAGCGAAGGAGAAGAGGTGCAGCAACGCGTGCCGTTGAGGGAGAGAGTGGGA GTGGTCAGATGTTACTCCGGTTCCCTCAAACGACGGCCCTAACCTGTCGTTCCGATCCAGTACA CTGAAGAGTTTTCCGAAGTTATGGATTACTTTTCGCGCCGTTTACCTCACCGATGAACGCTCCCCCT CGCGCCCTCGCTCTCACAGCCGAAGCCGTTCAATTCAACTCCGGCAACTACACTGTGTGGCATT CCGACGGTTGTTACTTGAGTCGCTAAAAGTCGACTTGAACGATGAACTGGAGTTTGTGGAGCGTA TGGCCGCTGGAAATTCTAAAAATTATCAGATGTGnATGTTCTGTAGGCATCCTAGACGATGGGTT GCCGAGAAGTTAGGTCTGAAGCTAGAAACAATGAGCTCGAGTTCACCAAAAAGATACTGTCCGT TGATGCCAAACATTATCATGCATGGTCTCATAGACAGTGGGCTCTTCAAACACTAGGAGGATGGG AAGATGAACCTTAATTATTGCACAGAACTACTTAAAGAAGACATTTTTAACAATTCTGCTTGGAA CAGAGATATTTTGTGATAACAAGGTCTCCTTTCTTGGGGGGCCTAAAAGCTATGAGAGAGTCTGA AGTGCTTTTACACCATCGAAGCCATTATAGCCTACCCTGAAAATGAAAGCTCGTGGAGATATCTAC GAGGACTTTATAAAGGTGAAACTACTTCATGGGTAAATGATCCTCAAGTTTCTTCAGTATGCTTA AAGATTTTGAAGAACTAAGAGCAACTACGTGTTTGCTCTTAGCACTATTTTAGATCTTATATGCTT TGGTTATCAACCAATGAAGACATTAGAGATGCCATTGACGCCTTAAAGACCGCAGATATGGATA AACAAGATTTAGATGATGATGAGAAAGGGGAACAACAAATTTAAATATAGCACGAAATATTTGT TCTATCCTAAAACAAGTTGATCCAATTAGAACCAACTATTGGATTTGGCGCAAGAGCAGACTTCC T

A disclosed FT4 polypeptide (SEQ ID NO:33) encoded by SEQ ID NO:31 has 347 amino acid residues and is presented in Table 7B using the one-letter amino acid code.

Table 7B. Encoded FT4 protein sequence (SEQ ID NO:33).

MESGSSEGEVQQRVPLRERVEVSDVTPVPQNDGPNPVVPIQYTEEFSEVMDYFRAVYLTDEERS PRALALTAEAQVFNNGNYTVWHFRRLLES LKVDLNDELEFVERMAAGNSKNYQMXMFCRHPRR WVAEKLGPPEARNELEFTKKILSVDAKHYHAWSHRQWALQTLGGWEDELNYCTELLKEDI FNNS AWNORYFVITRSPFLGGLKAMRESEVLYTIEAI IAYPENESSWRYLRGLYKGETTSWVNDPQVS SVCLKILRTKSNYVFALSTILDLCFGYQPNEDIRDAIDALKTADMDKQDLDDDEKGEQQNLNI ARNICSILKQVDPIRTNYWIWRKSRLP
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Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 8.

5 Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* alpha subunit of SEQ ID NO:31. The disclosed complimentary sequence is
10 shown as SEQ ID NO:32.

SEQ ID NO:32

AGGAAGTCTGCTCTTGCGCCAAATCCAATAGTTGGTTCTAATTGGATCAACTTGTTTTAGGATAGAACAAAT
ATTCGTGCTATATTTAAATTTTGGTTGCCCTTCTCATCATCTAAATCTTGTTTATCCATATCTGC
15 GGTCTTTAAGGCGTCAATGGCATCTCTAATGTCTTCATTTGGTTGATAACCAAAGCATATAAGATCTAAAT
AGTGCTAAGAGCAAACACGTAGTTGCTCTTAGTTCTCAAAATCTTTAAGCATACTGAAGAACTTGAGGATC
ATTTACCCATGAAGTAGTTTACCTTTATAAAGTCCTCGTAGATATCTCCACGAGCTTTCATTTTCAGGGTA
GGCTATAATGGCTTCGATGGTGTAAGCACTTCAGACTCTCTCATAGCTTTTAGGCCCCCAAGAAAGGAGA
CCTTGTTATGACAAAATATCTCTGATTCCAAGCAGAAATGTTAAAAATGTCTTCTTTAAGTAGTTCTGTGCA
20 ATAATTAAGTTTCATCTTCCCATCCTCCTAGTGTTTGAAGAGCCCACTGTCTATGAGACCATGCATGATAATG
TTTGGCATCAACGGACAGTATCTTTTTGGTGAACCTCGAGCTCATTGTTTCTAGCTTCAGGACCTAACTTCTC
GGCAACCCATCGTCTAGGATGCCTACAGAACATNCACATCTGATAATTTTGAATTTCCAGCGGCCATACG
CTCCACAAACTCCAGTTTCATCGTTCAAGTCGACTTTTAGCGACTCAAGTAACAACCGTCGGAATGCCACAC
AGTGTAAGTTGCCGGAGTTGAATTGAACGGCTTCGGCTGTGAGAGCGAGGGCGCGAGGGGAGCGTTTCATCGGT
25 GAGGTAAACGGCGCGAAAGTAATCCATAACTTCGGAAAACCTTCAGTGTACTGGATCGGAACGACAGGGTT
AGGGCCGTCGTTTTGAGGAACGGAGTAACATCTGACCACTCCACTCTCTCCCTCAACGGCACGCGTTGCTG
CACCTCTTCTCCTTCGCTAGACCCAGATTCCAT

Glycine max FTB

30 A disclosed nucleic acid of 1035 nucleotides (also referred to as FT5) is shown in Table 8A.

Table 8A. FT5 Nucleotide Sequence (SEQ ID NO:34).

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GCCACCATTCTCGCAACGCCCAAACCTCATGTTGGAGCTTCAACGCGATAATCACATGCAGTA
TGTCTCCAAAGGCCTTCGCCATCTCAGTTCCGCATTTTCCGTTTTGGACGCTAATCGACCCTGGC
TCTGCTACTGGATCTTCCACTCCATTGCTTTGTTGGGAGAATCCGTCGATGATGAACCTCGAAGAT
AACGCTATCGATTTTCTTAACCGTTGCCAGGATCCGAATGGTGGATATGCCGGGGGACCAGGCCA
GATGCCTCATATTGCCACAACCTTATGCTGCTGTTAATTCACCTATTACTTTGGGTGGTGAGAAAT
CCCTGGCATCAATTAATAGAGATAAACTGTATGGGTTTCTGCGGCGGATGAAGCAACCAAATGGT
GGATTGAGGATGCATGATGAAGGTGAAATTGATGTTTCGAGCTTGCTACACTGCCATTTCTGTTGC
AAGTGTTTTGAACATTTTGGATGATGAGCTGATCCAGAATGTTGGAGACTACATTATAAGCTGTC
AAACATATGAGGGTGGCATTGCTGGTGAGCCTGGTCTGAGGCTCATGGTGGGTACACCTTTTGT
GGATTAGCTACAATGATTCTGATTGGTGAGGTTAATCACTTGGATCTGCCTCGATTAGTTGACTG
GGTGGTATTCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAACAATAAACTGGTGGATGGAT
GCTATTCTTTTGGCAGGGAGGTGCTGTTGCTCTATTGCAAAGATTATCTTCTATTATCAACAAA
CAGATGGAAGAGACATCACAGATTTTTCGGTATCTTATGTATCTGAAGCAAAAGAAAGTTTGA
TGAACCTCTAGTCATGCAACATGCCGTGGTGAGCATGAAGGCACCAAGTGAATCCAGTTCATCTG
ATTTTAAAAATATTGCCTATAAATTTATTAATGAGTGGAGAGCACAAGAACCACCTTTTTCACAGT
ATTGCTTTACAGCAATATATTCTCTTATGTGCACAGGAGCAAGAGGGTGGATGAGAGACAAAAC
GGGTAAACGTAGAGATCATTATCACACATGTTACTGTTTAAAGTGGACTCTCATTGTGCCAGTATA
GTTGGTCAAAGCACCCAGATTCTCCACCAC

```

A disclosed FT5 polypeptide (SEQ ID NO:36) encoded by SEQ ID NO:34 has 378 amino acid residues and is presented in Table 8B using the one-letter amino acid code.

Table 8B. Encoded FT5 protein sequence (SEQ ID NO:36).

```

ATIPRNAQTLMLQLQDNHMQYVSKGLRHLSSAFSVLDANRPWLICYWIFHSIALLGESVDDELE
DNAIDFLNRCQDPNGGYAGGPGQMPHIATTYA AVNSLITLGGEKSLASINRDKLYGFLRRMKQP
NGGFRMHDEGEIDVRACYTAISVASVLNILDDELIQNVGDYIISCQTYEGGIAGEPGSEAHGGY
TFCGLATMILIGEVNHLDLRLVDWVVFROGKECGFQGRTNKLVDGCYSFWQGGAVALLQRLSS
IINKQMEETSQIFAVSYVSEAKESLDGTSSHATCRGEHEGTSESSSSDFKNIA YKFINEWRAQE
PLFHSIALQQYIILLCAQE QEGGLRDKPGKRRDHYHTCYCLSLCQYSWSKHPSPP :

```

5 Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 8.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid
10 amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* beta subunit of SEQ ID NO:34. The disclosed complementary sequence is shown as SEQ ID NO:35.

15 SEQ ID NO:35

```

GTGGTGGAGAATCTGGGTGCTTTGACCAACTATACTGGCACAATGAGAGTCCACTTAAACAGTAACATGTGT
GATAATGATCTCTACGTTTACCCGGTTTGTCTCTCAGTCCACCCTCTTGCTCCTGTGCACATAAGAGAATAT
ATTGCTGTAAAGCAATACTGTGAAAAAGTGGTTCTTGTGCTCTCCACTCATTAATAAATTTATAGGCAATAT
TTTTAAAATCAGATGAACCTGGATTCACTGGTGCCTTCATGCTCACACGGCATGTTGCATGACTAGAGGTTC
20 CATCCAAACTTTCTTTTGTCTTCAGATACATAAGATACCGCAAAAATCTGTGATGTCTCTTCCATCTGTTTGT
TGATAATAGAAGATAATCTTTGCAATAGAGCAACAGCACCTCCCTGCCAAAAGGAATAGCATCCATCCACCA
GTTTATTTGTTCTCCCTGGAATCCACATTCTTACCTTGTCGGAATACCACCCAGTCAACTAATCGAGGCA
GATCCAAGTGATTAACCTACCAATCAGAATCATTTGTAGCTAATCCACAAAAGGTGTACCCACCATGAGCCT

```


5 CAGAACCAGGCTCACCAGCAATGCCACCCTCATATGTTTGACAGCTTATAATGTAGTCTCCAACATTTCTGGA
 TCAGCTCATCATCCAAAATGTTCAAAACACTTGCAACAGAAATGGCAGTGTAGCAAGCTCGAACATCAATTT
 CACCTTCATCATGCATCCTGAATCCACCATTGGTTGCTTCATCCGCCGAGAAACCCATACAGTTTATCTC
 TATTAATTGATGCCAGGGATTTCTCACCACCCAAAGTAATAAGTGAATTAACAGCAGCATAAGTTGTGGCAA
 TATGAGGCATCTGGCCTGGTCCCCCGGCATATCCACCATTCCGATCCTGGCAACGGTTAAGAAAATCGATAG
 CGTTATCTTCGAGTTTCATCATCGACGGATTCTCCCAACAAAGCAATGGAGTGGAAGATCCAGTAGCAGAGCC
 AGGGTCGATTAGCGTCCAAAACGGAAAATGCGGAAGTGAAGTGGCGAAGGCCTTTGGAGACATACTGCATGT
 GATTATCGCGTTGAAGCTCCAACATGAGGGTTTGGGCGTTGCGAGGAATGGTGGC

10 Zea maize FTB

A disclosed nucleic acid of 1235 nucleotides (also referred to as FT6) is shown in Table 9A.

Table 9A. FT6 Nucleotide Sequence (SEQ ID NO:37).	
GCGGATCCCGACCTACCGAGGCTCACGGTGACGCAGGTGGAGCAGATGAAGGTGGAGGCCAGGG TTGGCGACATCTACCGCTCCCTCTTCGGGGCCGCGCCCAACACGAAATCCATCATGCTAGAGCTG TGGCGTGATCAGCATATCGAGTATCTGACGCCTGGGCTGAGGCATATGGGACCAGCCTTTCATGT TCTAGATGCCAATCGCCCTTGGCTATGCTACTGGATGGTTCATCCACTTGCTTTGCTGGATGAAG CACTTGATGATGATCTTGAGAATGATATCATAGACTTCTTAGCTCGATGTCAGGATAAAGATGGT GGATATAGTGGTGGACCTGGACAGTTGCCTCACCTAGCTACGACTTATGCTGCTGTAAATACACT TGTGACAATAGGGAGCGAAAGAGCATTGTCATCAATCAATAGGGGCAACCTGTACAATTTTATGC TGCAGATGAAAGATGTATCAGGTGCTTTCAGAATGCATGATGGTGGCGAAATTGATGTCCGTGCT TCCTACACCGCTATATCGGTTGCCAGCCTTGTGAATATTCTTGATTTTAAACTGGCAAAAGGTGT AGGCGACTACATAGCAAGATGTCAAACCTATGAAGGTGGTATTGCTGGGGAGCCTTATGCTGAAG CACATGGTGGGTATACATTCTGTGGATTGGCTGCTTTGATCCTGCTTAATGAGGCAGAGAAAGTT GACTTGCCTAGTTTGGATTGGCTGGGTGGCTTTTCGTCAAGGAGTGGAATGCGGATTTCAAGGACG AACTAATAAATTGGTTGATGGTTGCTACTCCTTTTGGCAGGGAGCTGCCATTGCTTTCACACAAA AGTTAATTACGATTGTTGATAAGCAATTGAGGTCTCGTATTCTGCAAAAGGCCATCAGGAGAG GATGCCTGCAGCACCAGTTCATATGGGTGCACCGCAATAAGTCTTCTCTGCTGTGGACTATGC GAAGTTTGGATTTGATTTTATACAACAGAGCAACCAATTGGCCCACTCTCCATAACATTGCC TGCAACAATACATCCTACTTTGTTCTCAGGTACTAGAGGGAGGCTTGAGGGATAAGCCTGGAAAG AACAGAGATCACTATCATTCATGCTACTGCCTCAGTGGCCTCGCAGTTAGCCAGTACAGTGCCAT GACTGATACTGGTTTCGTGCCCATACCTCAGCATGTGCTTGGACCGTACTCTAATTTGCTGGAGC CAATCCATCC	

15 A disclosed FT6 polypeptide (SEQ ID NO:39) encoded by SEQ ID NO:37 has 414
 amino acid residues and is presented in Table 9B using the one-letter amino acid code.

Table 9B. Encoded FT6 protein sequence (SEQ ID NO:39).	
ADPDLPRLTVTQVEQMKVEARVGDIIYRSLFGAAPNTKSIMLELWRDQHIEYLTPLRHMGPFAFH VLDANRPWL CYWMVHPLALLDEALDDLENDI IDFLARCQDKDGGYSGGPGQLPHLATTYA AVN TLVTIGSERALSSINRGNLYNFM LQMKDVSGAFRMDHGG EIDVRASYTAISVASLVNILD FKLA KGVGDYIARCQTYEGGIAGEPYAEAHGGYTFGLAALILLNEAEKVDLPSLIGWVAFRQGV ECG FQGRTNKLV DGCYSFWQGA AIAFTQKLITIVDKQLRSSYSCKRPSGEDACSTSSYGCTANKSSS AVDYAKFGFDFIQSNQIGPLFHNIALQQYILLCSQVLEGGRLDKPKGNRDHYHSCYCLSG LAV SQYSAMTDTGSCPLPQHVLGPYSNLL EPIH	

Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 8.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the
 5 *Zea maize* beta subunit of SEQ ID NO:37. The disclosed complementary sequence is shown as SEQ ID NO:38.

SEQ ID NO:38

```

10 GGATGGATTGGCTCCAGCAAATTAGAGTACGGTCCAAGCACATGCTGAGGTAATGGGCACGAACCAGTATCA
   GTCATGGCACTGTACTGGCTAACTGCGAGGCCACTGAGGCAGTAGCATGAATGATAGTGATCTCTGTTCTTT
   CCAGGCTTATCCCTCAAGCCTCCCTCTAGTACCTGAGAACAAAGTAGGATGTATTGTTGCAGGGCAATGTTA
   TGGGAAGAGTGGGCCAATTTGGTTGCTCTGTTGTATAAAATCAAATCCAAACTTCGCATAGTCCACAGCAGAG
   GAAGACTTATTCGCGGTGCACCCATATGAACTGGTGTGCAGGCATCCTCTCCTGATGGCCTTTTGCAGGAA
   TACGAGGACCTCAATTGCTTATCAACAATCGTAATTAACCTTTTGTGTGAAAGCAATGGCAGCTCCCTGCCAA
15 AAGGAGTAGCAACCATCAACCAATTTATTAGTTTCGTCTTGAAATCCGCATTCCACTCCTTGACGAAAAGCC
   ACCGAGCCAATCAAACCTAGGCAAGTCAACTTTCTCTGCCTCATTAAGCAGGATCAAAGCAGCCAATCCACAG
   AATGTATACCCACCATGTGCTTCAGCATAAGGCTCCCCAGCAATACCACCTTCATAAGTTTGACATCTTGCT
   ATGTAGTCGCCTACACCTTTTGCCAGTTTAAATCAAGAATATTCACAAGGCTGGCAACCGATATAGCGGTG
   TAGGAAGCACGGACATCAATTCGCCACCATCATGCATTCTGAAAGCACCTGATACATCTTTCATCTGCAGC
20 ATAAATTTGTACAGGTTGCCCTATTGATTGATGACAATGCTCTTTCGCTCCCTATTGTCACAAGTGTATTT
   ACAGCAGCATAAGTCGTAGCTAGGTGAGGCAACTGTCCAGGTCCACCACTATATCCACCATCTTTATCCTGA
   CATCGAGCTAAGAAGTCTATGATATCATTCTCAAGATCATCATCAAGTGCTTCATCCAGCAAAGCAAGTGGA
   TGAACCATCCAGTAGCATAGCCAAGGGCGATTGGCATCTAGAACATGAAAGGCTGGTCCCATATGCCTCAGC
   CCAGGCGTCAGATACTCGATATGCTGATCACGCCACAGCTCTAGCATGATGGATTTTCGTGTTGGGCGCGGCC
25 CCGAAGAGGGAGCGGTAGATGTCGCCAACCCTGGCCTCCACCTTCATCTGCTCCACCTGCGTCACCGTGAGC
   CTCGGTAGGTCTGGGATCCGCC
  
```

The FTA and FTB nucleic acids and amino acids disclosed above have homology
 30 to other members of the FT protein family (GenBank ID NOs: U63298, U83707, and
 U73203; WO 00/14207; Cutler et al., Science 273(5279):1239-41, 1996; Ziegelhoffer et
 al., Proc Natl Acad Sci U S A. 97(13):7633-8, 2000). The homology between these and
 other sequences is shown graphically in the ClustalW analysis shown in Tables 10A-10D.
 In the ClustalW alignment, the black outlined amino acid residues indicate regions of
 35 conserved sequence (*i.e.*, regions that may be required to preserve structural or functional
 properties), whereas non-highlighted amino acid residues are less conserved and can
 potentially be altered to a much broader extent without altering protein structure or
 function.

Table 10A. ClustalW Nucleic Acid Analysis of FT Alpha Subunits

- 1) BNA-12; FT2 (SEQ ID NO:6)
- 2) At-FT-A; FT1 (SEQ ID NO:1)
- 3) PPI-Soy-FTA; FT4 (SEQ ID NO:31)
- 4) Pea-FT-A (SEQ ID NO:59)
- 5) Tomato-FTA (SEQ ID NO:60)
- 6) Rice-FT-A (SEQ ID NO:61)

- 360 370 380 390 400 410 420
- BnA-12 GCTTAATAACGACTTCTATGAAGAGCTCAAGTTTCATCGAAGGCATTGCTGAGGATAACTCTGAAGAACTAC
- At-FT-A CTTTAATCAGCACTTCTTTGAAGAAGCTCGGATTCATCGAAGGCATTGCTGAGGATAACTCTGAAGAACTAC
- PPI-Sov-FTA GCTAAAGAGTCGACTTCAACGATGAAGCTGGAGTTGTGTGAGCGGTATGGCCCTCGGAATATTCAAAAATTAT

Pea-FT-A	ACTGAAAGTTGACCTACATGTTGAACGGGAATTCGTGGAGCGCTTTGCCAGTGGCAATTCAAAAAATTAT
Tomato-FTA	ATTTGGGTGTTGATTTACGTGAAGAATTGAAGTTTGTGATCGCATTTGCTGGGAGCAATACAAAAATTAT
Rice-FT-A	ACTGGATGCTGATCTGCGTGAGGAATGGATTTTGTGGACCGAATTGTGCAATGTAACCAAAAAATTAT
Zea mays-FT-A	ACTAGATTTTGATTTACTAGAGGAGATGAAATTTGTGCAAAAAATTGCTGAATGCAATCCAAAAATTAC
Soy1-FT-A	GCTAAAAGTCGACTTGAACGATGAACCTGGATTTTGTGGAGCGTATGGCCCTGGAAATTCAAAAATTAT
Soy2-FT-A	GCTAAAAGTCGACTTGAACGATGAACCTGGAGTTTGTGGAGCGTATGGCCCTGGAAATTCAAAAATTAT
Triticum-FT-A	ACTGGATGCTGATTTATTGCTAGAAATGCATTTTGTGGACCAATTGCTGAATCTAATCAAAAAATTAC
	430 440 450 460 470 480 490
BnA-12	CACTTGTGG-----CATCATCGACGATGGGTGCGCAGAGA
At-FT-A	CACTTGTGG-----CATCATCGGCGGATGGGTGCGCAGAGA
PPI-Soy-FTA	CAGATGTGN---ATGTTCTG-----TAG-----GCATCTAGACGATGGGTGCGCAGAGA
Pea-FT-A	CAGATTTGG-----CATCATAGACGATGGGTGCGTGAAGA
Tomato-FTA	CAAAATATGG-----CATCATAGACGCTGGCTTGCTGAGA
Rice-FT-A	CAAAATCTGG-----CATCAAGAGATGGCTTGCGGAGA
Zea mays-FT-A	CAAAATCTGG-----CACCATAGAGATGGCTTGCTGAGA
Soy1-FT-A	CAGATGTGG-----CATCATAGACGATGGGTGCGCAGAGA
Soy2-FT-A	CAGATGTGGCTGTGATGCTCTGCTCTGCTCTTTCTTCCATACTTTG
Triticum-FT-A	CACTTGTGG-----CATCAAGAGATGGCTTGCTGAGA
	500 510 520 530 540 550 560
BnA-12	AATGCGGTCTGATGTTGCGAGGAAGGGAAGTTGAGTTTACTCGGAGGGTACTATCACTTGATGCCAAGCA
At-FT-A	AATGCGGTCTGATGTTGCGAGGAGAGAACTTGAAATTTACCGGTAGAGTACTTTCACTTGATGCCAACA
PPI-Soy-FTA	AGTTAGGTCTGAGCTAGAAACAATGAGCTCGAGTTACCAAAAAGATAGTGTGCGTTGATGCCAACA
Pea-FT-A	AATTAGGACCTGAGCTAGAAACAAGTGAAGTTGAGTTTACCAAAAAGATAGTGTGCTGTTGACGCCAACA
Tomato-FTA	AGCTGGGAGCTGATGCTGTGACAAATGAGCTAGAAATTCACCAAGAAAATATTTTCTCAGGATGCAAAAA
Rice-FT-A	AATTAGGACCTGATGTTGCAAAATAGAGCAGCAATTTACAAGGAAGATAGTGTGATGCTGCAAAAA
Zea mays-FT-A	AATTAGGACCTGATGTTGCAAAACAAGAGCAGTGAATTCACAAATGAAGTACTTGTGTTGATGCAAAAA
Soy1-FT-A	AGTTAGGTCTGAGCTAGAAACAATGAGCTCGAGTTACCAAAAAGATAGTGTGCGTTGATGCCAACA
Soy2-FT-A	AGTTAGGTCTGAGCTAGAAACAATGAGCTCGAGTTACCAAAAAGATAGTGTGCGTTGATGCCAACA
Triticum-FT-A	AATAGGACCTGAGCTGCAAAATAGTGAACATGACTTCACAAAGGAAGATAGTGTGATGCTGCAAAAA
	570 580 590 600 610 620 630
BnA-12	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTAAGTAC
At-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTCGATTAC
PPI-Soy-FTA	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTAAGTAT
Pea-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTCAGTTAT
Tomato-FTA	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTGCCTAT
Rice-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTGCCTAT
Zea mays-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTAAGTAT
Soy1-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTAAGTAT
Soy2-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTAAGTAT
Triticum-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCGTTACAAGCATTAGGAGGATGGGAAGATGAAGCTTAAGTAT
	640 650 660 670 680 690 700
BnA-12	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCAATGGAATCAGAGGATTTACGTTTATAACTA
At-FT-A	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCAATGGAATCAGAGGATTTATGTCATCAACC
PPI-Soy-FTA	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
Pea-FT-A	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
Tomato-FTA	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
Rice-FT-A	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
Zea mays-FT-A	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
Soy1-FT-A	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
Soy2-FT-A	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
Triticum-FT-A	TGCAACGAGCTCTTGAAGCTGACGTTCTTTAAACAATCTGCTTGAATCAGAGATATTTTGTCTATAACAA
	710 720 730 740 750 760 770
BnA-12	GATGACCTTCGTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACAGTCAAGCCATTIT
At-FT-A	AATCTCCTTTGTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACATCAAGCCATTIT
PPI-Soy-FTA	GGTCTCCTTTCTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACATCAAGCCATTAT
Pea-FT-A	GGTCTCCTTTCTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACATCAAGCCATTAT
Tomato-FTA	GATGACCTTCGTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACAGTCAAGCCATTIT
Rice-FT-A	GATGACCTTCGTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACAGTCAAGCCATTIT
Zea mays-FT-A	GATGACCTTCGTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACAGTCAAGCCATTIT
Soy1-FT-A	GGTCTCCTTTCTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACATCAAGCCATTAT
Soy2-FT-A	GGTCTCCTTTCTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACATCAAGCCATTAT
Triticum-FT-A	GATGACCTTCGTTGGGAGGCTTAGAAGCCATGAGAGATCTGAAGTAAGCTACACAGTCAAGCCATTIT
	780 790 800 810 820 830 840
BnA-12	AGCAAAATCCCGGGAACGAGAGCTCTTGGAGGTACCTGAAAGCCCTTTACAAAGACGACAGAGTCTTGG
At-FT-A	AGCAAAATCCCGGGAACGAGAGCTCTTGGAGGTACCTGAAAGCCCTTTACAAAGACGACAGAGTCTTGG
PPI-Soy-FTA	AGCTACCTCTGAAATGAAAGCTCTTGGAGATATCTACAGGACTTTTAAAGGTGAAACTACTTCAATGG

Pea-FT-A TTCTTACCCAGAAATGAAAGCTCATGGAGATATCTTCGAGGACTTTTCAAAAGATGAATCCACCTATAT
 Tomato-FTA AGCTAGTCCAGACAATGAAAGCTCTGGAGGTATCTTCGTGGTCTTTACAAGAAATGATACACATCTCTA
 Rice-FT-A GGCTAACCCCTCAAAATGAAAGCCCTGGAGATACCTCAAAGGCCCTGTACAAGGGTGAAAATAACCTGGCTG
 Zea mays-FT-A AGCAAGGCTCAGAATGAAAGCCCTGGAGGTACCTCAAGGGTCTATACAAGGGTGAGAAATAACCTGCTA
 Soy1-FT-A AGCCTACCCTGAAATGAAAGCTCTGGAGATATCTACGAGGACTTTATAAAGGTGAAACTACTTCATGG
 Soy2-FT-A AGCCTACCCTGAAATGAAAGCTCTGGAGATATCTACGAGGACTTTATAAAGGTGAAACTACTTCATGG
 Triticum-FT-A GGTGAACCCCTCAAAATGAAAGCCCTGGAGATACCTCAGAGGTTTATATAAGGATGATAACAATTTGCTG

850 860 870 880 890 900 910
 BnA-12 ATTAGTGATCCAAAGTGTTCCTCAGTCTGTTTGAAGTCTCTCACGCGCGGACTGCTTCCATGGAATTCG
 At-FT-A ATTAGTGATCCAAAGTGTTCCTCAGTCTGTTTGAAGTCTCTCACGCGCACAGATTGCTTCCATGGAATTCG
 PPI-Soy-FTA GTAAATGATCCTCAAGTTTCTTCAGTATGCTTAAAGATTTTGAGAACTAAGAGCAAC---TACGTGTTTG
 Pea-FT-A GTAAATGATGCCAAAGTATCTTCTATGTTTAAAGATTTTGAGAACTAAGAGCAAC---TATTTGTTTG
 Tomato-FTA GTTCAGGATTCTCAAGTAGCATCACTACTTTGGGACGTCTTAACTCCCAAAATAGT---CATGTGCACG
 Rice-FT-A ATGGCTGATGAGCGCATCTCTGATGTTTGTCTCAAGGTCTTGAAACATGATTCCGACC---TGCCTATTGG
 Zea mays-FT-A GTAGAGGACGAGCGCATCTCTGCTGTTTGTGTTTCAAGGTCTTGAGAAATGATTGCAAC---TGTGTATTGG
 Soy1-FT-A GTAAATGATCCTCAAGTTTCTTCAGTATGCTTAAAGATTTTGAGAACTAAGAGCAAC---TACGTGTTTG
 Soy2-FT-A GTAAATGATCCTCAAGTTTCTTCAGTATGCTTAAAGATTTTGAGAACTAAGAGCAAC---TACGTGTTTG
 Triticum-FT-A GTGGCTGATAATGCATTTCTGATGCTTGCTCAAGGTCTTGAAATAGGATTGCAACA---TGCCTATTGG

920 930 940 950 960 970 980
 BnA-12 CTCTGAGCACCTTTTGGATCTTCTGTCGATGGGT---TGAGACCAACCAACGAGCATAGAGACTGGTGA
 At-FT-A CTCTGAGCACCTTTTGGATCTTCTATGATGGAC---TGAGACCAACCAACGAGCATAGAGACTCAGTGA
 PPI-Soy-FTA CTCTTAGCACTATTTTATGATCTTATGCTTTGGTTAT---CAACCAATGAAGACATTAGAGATGCCATTG
 Pea-FT-A CTCTTAGCACTATTTTATGATCTTATGCTTTGGTTAT---CAACCAATGAAGATTTTCAAGATGCCATTG
 Tomato-FTA CTCTGAGGTTCTTGTGGATCTTCTTGTGATGATT---TGGAACCGAGCCAAGAAATGAAAGTGTCTGAG
 Rice-FT-A CTCTGAGGTTCTTGTGGATCTTCTTGTGATGATT---TGGAACCGAGCCAAGAAATGAAAGTGTCTGAG
 Zea mays-FT-A CTCTTAGCACTATTTTATGATCTTATGCTTTGGTTAT---CAACCAATGAAGACATTAGAGATGCCATTG
 Soy1-FT-A CTCTTAGCACTATTTTATGATCTTATGCTTTGGTTAT---CAACCAATGAAGACATTAGAGATGCCATTG
 Soy2-FT-A CTCTTAGCACTATTTTATGATCTTATGCTTTGGTTAT---CAACCAATGAAGACATTAGAGATGCCATTG
 Triticum-FT-A CTCTTAGCACTATTTTATGATCTTATGCTTTGGTTAT---CAACCAATGAAGACATTAGAGATGCCATTG

990 1000 1010 1020 1030 1040 1050
 BnA-12 AAGCTCTAG---CT---AATGAAGA---A---CCAGAGAC
 At-FT-A AAGCTCTAG---CT---AATGAAGA---A---CCAGAGAC
 PPI-Soy-FTA AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT
 Pea-FT-A AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT
 Tomato-FTA AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT
 Rice-FT-A AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT
 Zea mays-FT-A AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT
 Soy1-FT-A AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT
 Soy2-FT-A AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT
 Triticum-FT-A AAGCCTTAAAGACCGGAGATATGGATAAACAAGATTAGATGATGATGAGAAAGGGGAACAACAATTT

1060 1070 1080 1090 1100 1110 1120
 BnA-12 TAACTTGGCCAAATTTGGTGTGATCAATCTGTGTGCTGTGATCCAAATGAAGGCTAACTATTGGGCATGG
 At-FT-A TAACTTGGCCAAATTTGGTGTGATCAATCTGTGTGCTGTGATCCAAATGAAGGCTAACTATTGGGCATGG
 PPI-Soy-FTA AAATATAGCAGCAAAATATTTGTTCTATCTTAAACAAGTTGATCCAAATGAGAACCACTATTGGATTGG
 Pea-FT-A AGATATAGCAGCAAAATATTTGTTCTATCTTAAACAAGTTGATCCAAATGAGAACCACTATTGGATTGG
 Tomato-FTA AGCACTGACAAAGAAATTTGTTCTATGTTGCAACATGCTGATCCAAATGAGAACCACTATTGGATTGG
 Rice-FT-A TGATCTTGGGACTGCAATCTGCTCAATATGCAAGAGATGTGATCCCTGCGGATAAATTAATGGTCTGG
 Zea mays-FT-A TGATCTTGGGACTGCAATCTGCTCAATATGCAAGAGATGTGATCCCTGCGGATAAATTAATGGTCTGG
 Soy1-FT-A AAATATAGCAGCAAAATATTTGTTCTATCTTAAACAAGTTGATCCAAATGAGAACCACTATTGGATTGG
 Soy2-FT-A AAATATAGCAGCAAAATATTTGTTCTATCTTAAACAAGTTGATCCAAATGAGAACCACTATTGGATTGG
 Triticum-FT-A TGATATTGAGTAGCTGTCTGCTCAATCTGCAAGAAATGTGATCCCTGCGGATAAATTAATGGTCTGG

1130 1140 1150 1160 1170 1180 1190
 BnA-12 ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 At-FT-A ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 PPI-Soy-FTA ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 Pea-FT-A ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 Tomato-FTA ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 Rice-FT-A ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 Zea mays-FT-A ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 Soy1-FT-A ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 Soy2-FT-A ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA
 Triticum-FT-A ACGAAGAGCAGATTACA---GTGGC-AGCAATTGAATATGTGACGCCCCAAAATCACACTTGAAAAA

1200 1210 1220 1230 1240 1250 1260
 BnA-12 GACCTTGATTAT---TAGT-TTTTACGT---AATTAAGTCTGCTAATTTATGAATCACATG-TTCAT
 At-FT-A GACCTTGATTAT---TAGT-TTTTACGT---AATTAAGTCTGCTAATTTATGAATCACATG-TTCAT
 PPI-Soy-FTA GACCTTGATTAT---TAGT-TTTTACGT---AATTAAGTCTGCTAATTTATGAATCACATG-TTCAT

Pea-FT-A GTCTATTGGAATTTGACGTCATGGAT-----AACAGGGTGGTGGTTTGTGTTATGATAT-GTTTT
Tomato-FTA GTGTTCAAGAA--TGAC-TTGTGAGA-----ATATTGTAAGTGGTTACGAAATACATA-CCTGC
Rice-FT-A TCATAATGGAGATATCTTCTAT-----CTTCGGTGTGA-----TTCTG
Zea mays-FT-A CTGTGAGATAGACATGTTTATGATAGTTTCATTGGACACCCAAACAGAGCGGACAGAGTGTATGGCTGCT
Soy1-FT-A GTTATGTGTAACTTAGT-TTATTGA-----AACTGGATTTTATT--ATTATTATT-TTTAT
Soy2-FT-A GTTATGTGTAACTTAGT-TTATTGA-----AACTGGATGTTTATT--ATTATTATT-TTTAT
Triticum-FT-A GCATGATGTAACTCAATCGAAGGGGTT-----GACGCAGTGTATGAAAACCT--TTCC

1270 1280 1290 1300 1310 1320 1330
....|....|....|....|....|....|....|....|....|....|....|....|....|....|

BnA-12
At-FT-A GT-TAACATGTATCAAAACAATCTTGATTCTCAAAAAAAAAAAAAAAAAAAAAA-----
PPI-Soy-FTA
Pea-FT-A CC-AGATGTAATTCTATATTTAACAGCAAAGTTGTTTAAACATGGTGTTAACAAACCAATGATCTCCAA
Tomato-FTA ATCTAAGGTGATCCTTCGGGCACATGTGCTGGGAAGTGACTGAATATCACGAAGAACTAAAAAACTGTG
Rice-FT-A GGCCTTGAGGTGCT--AGCTACATTTGTTATGAACTTTCTTGGGCATAACTGATCACTGATATTAC
Zea mays-FT-A ACCTTCTCCGTGACTGAAAGCAGTGCTTGTAAACG--TTTTGTTTAGTAAAAATTTGTGAGTCTTACTGCT
Soy1-FT-A GT-TGTCATGTATCTGTTGT---GCAAATTT---ATCTTTTGTGTCATGCCATTACTGGCATTGGA
Soy2-FT-A GT-TGTCATGTATCTGTTGT---GCAAATTT---ATCTTTTGTGTCATGCCATTACTGGCATTGGA
Triticum-FT-A GTGATCTTGGTGCAG--AGCAA--TTTGACTGA--TTTACTGGGAAAAATCAATCATGACATGCATG

1340 1350 1360 1370 1380 1390 1400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|

BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A AAAATCAATGTTTATTTCTCTTCATTTGCTGATTTGTGGCATTAACATTCTTGATGAT-TTTGTGGTA
Tomato-FTA ATTGGCAACATTGTACTACTCCAAATAGGTCACCTTCGATGACTTTTGTACTGCCTTGA-GTTTTGGCT
Rice-FT-A TCCAATATTGTGTTCTAAA-----
Zea mays-FT-A CCAAACACACCTTATGCAACCATATTTGAATAT---TTCACATGTAAGCT---TGA-----A-TC
Soy1-FT-A GTG--TAAGGATGAAAGCCATGCA-----GAATAAGAAATTTAAGTTTTTT---TTTCCGTTG
Soy2-FT-A GTG--TAAGGATGAAAGCCATGCA-----GAATAAGAAATTTAAGTTTTTT---TTTCCGTTG
Triticum-FT-A CCCAACAAATGCTTGTGTGAATATGTTACTGCCTGATATTCACATGTTAGCAGAATGAGAATAACCAATC

1410 1420 1430 1440 1450 1460 1470
....|....|....|....|....|....|....|....|....|....|....|....|....|....|

BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A AAAAAAAAAAAAAAAAAAAAAA-----
Tomato-FTA CTGCTATGTTTTGTAACTTTTGGATATGGATGCATAGCTTATTGATACCTTTTGGTGACTTAAATACTCT
Rice-FT-A
Zea mays-FT-A CAGGTGTGTTTGTAAATGTATTACACTT--G-CCATGGGAGCCTAAATGAGACCCATAATCACTTCCACT
Soy1-FT-A AAAAA-----
Soy2-FT-A AAAAAAAAAAAAAAAAAAAAAA-----
Triticum-FT-A AAACCTCAACGAGCAGATTGTTACAGTAACGGCCACTGGTGGTGTGAAATCCTGAAATCTGCTTCAGTC

1480 1490 1500 1510 1520 1530 1540
....|....|....|....|....|....|....|....|....|....|....|....|....|....|

BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A
Tomato-FTA GGAAGGCAGGTAGCATGTGTATAATTCAGTGTTACTTCCCATGTCGAGTTAGATGCTTGAAAATTTTAGT
Rice-FT-A
Zea mays-FT-A AGAGTCGGAAGACCGT-GTCGAGCAGTTCACTCATATGGTCACTTAAAGCAAAAAAAAAAAAAAAAAAAAAA
Soy1-FT-A
Soy2-FT-A
Triticum-FT-A ACTTTGCCTTGTATTACAGTTGAGTCTGTTGTGTGATCTGTACCTAATGCATGTACACAATCATCAATT

1550 1560 1570 1580 1590 1600 1610
....|....|....|....|....|....|....|....|....|....|....|....|....|....|

BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A
Tomato-FTA AGGTGTTCTTTTATGAAGCACACATTAATGTGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Rice-FT-A
Zea mays-FT-A
Soy1-FT-A
Soy2-FT-A
Triticum-FT-A ATTAGTTTTGTACCAATGAGTATTTCATGAAAAA

BnA-12
At-FT-A
PPI-Soy-FTA

Pea-FT-A -
 Tomato-FTA A
 Rice-FT-A -
 Zea mays-FT-A -
 Soy1-FT-A -
 Soy2-FT-A -
 Triticum-FT-A -

Table 10B. ClustalW Amino Acid Analysis of FT Alpha Subunits

- 1) BNA-12; FT2 (SEQ ID NO:7)
- 2) At-FT-A; FT1 (SEQ ID NO:5)
- 3) PPI-Soy-FTA; FT4 (SEQ ID NO:33)
- 4) Pea-FT-A (SEQ ID NO:66)
- 5) Tomato-FTA (SEQ ID NO:67)
- 6) Rice-FT-A (SEQ ID NO:68)
- 7) Zea mays-FT-A (SEQ ID NO:69)
- 8) Soy1-FT-A (SEQ ID NO:70)
- 9) Soy2-FT-A (SEQ ID NO:71)
- 10) Triticum-FT-A (SEQ ID NO:72)

	10	20	30	40	50	60	70
BnA-12	MDYFRATYFSDERS	ALRAL
At-FT-A	-----	MNFDETVPLSQPLEWS	SDVVPETCCDGN	NPVVP	IAYKEEFRET	MDYFRATYFSDERS	SPRALR
PPI-Soy-FTA	-----	MESGSSEGEVQQRVPLRE	FVEWS	SDVTPVPQNDGPN	PVVPIQYTEEFSE	VMDYFRAYVLT	TERS
Pea-FT-A	-----	MAGNIEVEE-DDRVPLRLPEWS	SDVTPPCDDGPS	PPVVPINYSSEFSE	VMDYFRAYVFAKELSS	RALA	
Tomato-FT-A	-----	MDSCVTKTRIPFKE	RPPWADVKVP	PCDDGCP	PPVPIAYTEB	ESFSTMDYFRAYVAD	ERS
RiceFT-A	-----	MAPSSTSSEGASDEWLPPSRPE	LADVVVPVTCDDGPH	PPVVALAYRDEE	REVM	MDYFRATYF	FGERS
Zea mays-FT-A	-----	MEHTLSGGPSSWPELA	DVVPVPQNDGGS	PPVVS	IAYRDEERG	VM	DMYFRATYF
Soy1-FT-A	-----	MESGSSEGEVQQRVPLRE	FVEWS	SDVTPVPQNDGPN	PVVPIQYTEEFSE	VMDYFRAYVLT	TERS
Soy2-FT-A	-----	MESGSSEGEVQQRVPLRE	FVEWS	SDVTPVPQNDGPN	PVVPIQYTEEFSE	VMDYFRAYVLT	TERS
Triticum-FT-A	-----	DVAPLPCADGCP	PPVVS	IAYRDEE	REVM
	80	90	100	110	120	130	140
BnA-12	LTEEDRLNSGNYTVWHFGRLLVLEELNNDLYEELKFTETESIAEDNSKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
At-FT-A	LTEETRLNSGNYTVWHFRRLVLEALNHDLFELEETERIAEDNSKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
PPI-Soy-FTA	LTAEAVQNSGNYTVWHFRRLVLESLSKVDLNDELEFVERMAAGNSKNYCLW	-----	-----	-----	-----	MFCR	HPRRWVA
Pea-FT-A	LTAEAICLNSGNYTVWHFRRLVLESLSKVDLHVEREFVERMAAGNSKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
Tomato-FT-A	LTEGAILQNLPGNYTVWHFRRLVLEALGVDLREDLKFFVBRIAGENPKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
RiceFT-A	LTAEVLDLNPNGNYTVWHFRRLVLEALDADLREMDFVDRIVECNPKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
Zea mays-FT-A	LTAEAELNPGNYTVWHFRRLVLESLODLDLEEMKFVELIAECNPKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
Soy1-FT-A	LTAEAVQNSGNYTVWHFRRLVLESLSKVDLNDELEFVERMAAGNSKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
Soy2-FT-A	LTAEVQVNSGNYTVWHFRRLVLESLSKVDLNDELEFVERMAAGNSKNYCLW	-----	-----	-----	-----	-----	HHRRWVA
Triticum-FT-A	LTADAILNPGNYTVWHFRRLVLEALDADLLEEMHFVDCIAESNPLNYCLW	-----	-----	-----	-----	-----	HHRRWVA
	150	160	170	180	190	200	210
BnA-12	EKLGPDVAGLEKEFTRRVLSLDAKH	YAWSHRQWALQALGGWENELNYCHELLEADVFNN	SAWNQRYFVI				
At-FT-A	EKLGPDVAGLEKEFTRRVLSLDAKH	YAWSHRQWTLRQALGGWEDELNYCHELLEADVFNN	SAWNQRYFVI				
PPI-Soy-FTA	EKLGPPEARNNLELEFTKKILSVDAKH	YAWSHRQWALQALGGWEDELNYCHELLEADVFNN	SAWNQRYFVI				
Pea-FT-A	EKLGPPEARNNLELEFTKKILSVDAKH	YAWSHRQWVLCALGGWEDELNYCHELLEADVFNN	SAWNQRYFVI				
Tomato-FT-A	EKLGPADAVTNELEFTKKILSVDAKH	YAWSHRQWVLCALGGWEDELA	YCCOLLEEDVFNN	SAWNQRYFVI			
RiceFT-A	EKLGPDIANKHEFTTKILSVDAKH	YAWSHRQWVLCALGGWETELQYCNOLLEEDVFNN	SAWNQRYFVI				
Zea mays-FT-A	EKLGPDIANKHEFTTKILSVDAKH	YAWSHRQWVLCALGGWETELQYCNOLLEEDVFNN	SAWNQRYFVI				
Soy1-FT-A	EKLGPPEARNNLELEFTKKILSVDAKH	YAWSHRQWALQALGGWEDELNYCHELLEADVFNN	SAWNQRYFVI				
Soy2-FT-A	EKLGPPEARNNLELEFTKKILSVDAKH	YAWSHRQWALQALGGWEDELNYCHELLEADVFNN	SAWNQRYFVI				
Triticum-FT-A	EKLGPPEARNNLELEFTKKILSVDAKH	YAWSHRQWVLCALGGWESLQYCNOLLEEDVFNN	SAWNQRYFVI				
	220	230	240	250	260	270	280
BnA-12	TRSPSLGGL	EAMRESEVSYTKATIANP	GNES	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	SRADCFHG	
At-FT-A	TRSPSLGGL	EAMRESEVSYTKATIANP	GNES	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	SRADCFHG	
PPI-Soy-FTA	TRSPFLGGL	EAMRESEVLYTEAT	IAYPENESS	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	RTKSNYV	
Pea-FT-A	TRSPVLGGL	EAMRESEVLYTEAT	IAYPENESS	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	RTKSNYV	
Tomato-FT-A	TRSPFLGGL	EAMRESEVLYTEAT	IAYPENESS	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	RTKSNYV	
RiceFT-A	TRSPFLGGL	EAMRESEVLYTEAT	IAYPENESS	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	RTKSNYV	
Zea mays-FT-A	TRSPFLGGL	EAMRESEVLYTEAT	IAYPENESS	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	RTKSNYV	
Soy1-FT-A	TRSPFLGGL	EAMRESEVLYTEAT	IAYPENESS	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	RTKSNYV	
Soy2-FT-A	TRSPFLGGL	EAMRESEVLYTEAT	IAYPENESS	SWRYLRGLYKGETTSWNL	POVSSVCLKVL	RTKSNYV	

Triticum-FT-A	TRSPILGGLAAMRSEVDYTYEAIMVNFQNESPWRYLRGLYKDDNNLLVADNRHSDACLKVLI-NKDWTCTV
	290 300 310 320 330 340 350
BnA-12	FALSTLLDLLCDGLRPINEHRDSVKALANEEPTN-----LANLVCHILCRVDPIRAN
At-FT-A	FALSTLLDLLCDGLRPINEHKDSVRALANEEPTN-----LANLVCHILGRVDPIRAN
PPI-Soy-FTA	FALSTLLDLLCFGYOPNEDIRDAIBALKTADM--DKQDLDDDEKGEQQLNLTARNICSIILKQVDPIRTN
Pea-FT-A	FALSTLLDLLSASVHOPNEDIRDAIBALKLQIL--IKQ--DSE-----TAITICSIILKQVDPIRVN
Tomato-FT-A	HALRFLLDLLCHDLPSOELKSAMVLTPOSC--SPD-----LATKKICSILEHADPMFVK
RiceFT-A	FALSLLDLLLQIGLOPSDEELKGTIEATKNSDPEADEAVDA--P-----PATAICSIILORCDPIPIN
Zea mays-FT-A	FALSLLDLLLCTGLOPSDEGLRSTEGTIRSSHPTADD--P-----PAAAVCCILKQCDPIAVN
Soy1-FT-A	FALSTLLDLLCFGYOPNEDIRDAIBALKTADM--DKQDLDDDEKGEQQLNLTARNICSIILKQVDPIRTN
Soy2-FT-A	FALSTLLDLLCFGYOPNEDIRDAIBALKTADM--DKQDLDDDEKGEQQLNLTARNICSIILKQVDPIRTN
Triticum-FT-A	FALSFLDLLRMGLOPSNELKGTIEAMENSDEP--ETGHA--P-----TAVAVCSILKQCDPIPIN
	360 370
BnA-12	YWAANKL-----
At-FT-A	YWAWRKSKITVAAI-----
PPI-Soy-FTA	YWIWRKSRIP-----
Pea-FT-A	YVWVRKSRIPQAA-----
Tomato-FT-A	YWNWRKSMRVQLLSQNAERLANLSVQE
RiceFT-A	YWSWYRTTSSQT-----
Zea mays-FT-A	YWSWFRDTSQIS-----
Soy1-FT-A	YWIWRKSRIPLSA-----
Soy2-FT-A	YWIWRKSRIPLSA-----
Triticum-FT-A	YWSWYRTTSS-----

Table 10C. ClustalW Nucleic Acid Analysis of FT Beta Subunits

- 1) PPI-BnFTb; FT3 (SEQ ID NO:8)
- 2) eral (SEQ ID NO:73)
- 3) Wiggum (SEQ ID NO:74)
- 4) PPI-Soy-FTB; FT5 (SEQ ID NO:34)
- 5) DuP-Soy-FTB (SEQ ID NO:75)
- 6) PPI-Corn-FTB; FT6 (SEQ ID NO:37)
- 7) DuP-Corn-FTB (SEQ ID NO:76)
- 8) Pea-FT-B (SEQ ID NO:77)
- 9) Tomato (SEQ ID NO:78)
- 10) Tobacco (SEQ ID NO:79)

	10 20 30 40 50 60 70
PPI-BnFTb	-----
eral	-----
Wiggum	ATGCCAGTAGTAACCCGCTTGATTCGTTTGAAGTGTGTAGGGCTCAGACTTGACCGGAGTGGACTCAATC
PPI-Soy-FTB	-----
DuP-Soy-FTB	-----
PPI-Corn-FTB	-----
DuP-Corn-FTB	-----
Pea FT-B	-----
Tomato	-----GTAAACGAGCGTTGATTT
Tobacco	-----
	80 90 100 110 120 130 140
PPI-BnFTb	-----
eral	-----
Wiggum	GGCGAATCTGTACGGAGGACACGGGAATCAACGCGGCGGAGAGTGATGGAAGAGCTTTCAAGCCTAAC
PPI-Soy-FTB	-----
DuP-Soy-FTB	-----
PPI-Corn-FTB	-----GGCGGATCCCCGACCTACCGAGGCTCAC
DuP-Corn-FTB	-----GGCGGATCCCCGACCTACCGAGGCTCAC
Pea FT-B	-----CGGACCCCCCGTCCACAATCGTGAT
Tomato	GTCGCTGACGAAATTTACAGTCAAGAGTAGTAACCGGTTGTAGTGAAAAAATGGAGTCGAGGAAAGTGAC
Tobacco	-----GGCACGAGCGGC--AC
	150 160 170 180 190 200 210
PPI-BnFTb	-----
eral	-----

Wiggum CGTGAGTCAGCGCGAGCAATTTCTGGTGGAGAACGATGTGTTCGGGATCTATAATTACTTCGACGCCAGC
PPI-Soy-FTB -----GCCACCATTTC
DuP-Soy-FTB -----GCCACCATTTC
PPI-Corn-FTB GGTGACGCGAGGTGGAGCAGATGAAGGTGGAGGCCAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCC
DuP-Corn-FTB GGTGACGCGAGGTGGAGCAGATGAAGGTGGAGGCCAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCC
Pea FT-B GATGACGTCTCCGCGAGCATTTCAACAACAGTTACTCAAACCACCGCGGAGTAACACATGGAAAGCTTCA
Tomato GAAGACGCTGGAAGATCAATGGGTGGTGGAGCGTCGAGTCCGAGAGATATACGATTATTTCTACAGCATT
Tobacco GAGGACACTGGAAGATCAATGGATGGTGGAGCGTCAAGTTCGGGAGATATACAATTTTTCTACAGCATT

220 230 240 250 260 270 280
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

PPI-BnFTb -----ATGGGAGATTTCAGCGAGATAAGCAATTGGATTATC-----TGATGA
eral -----TGATGA
Wiggum GACGTTTCTACTCABAAATACATGATGGAGATTTCAGCGAGATAAGCAATTGGATTATC-----TGATGA
PPI-Soy-FTB CTGCGAACCGCCAAACCCCTCAT-GTTGGAGCTTCAACCGCGATAATCAGAT-GCAGTAT-----GCTCCCA
DuP-Soy-FTB CTGCGAACCGCCAAACCCCTCAT-GTTGGAGCTTCAACCGCGATAATCAGAT-GCAGTAT-----GCTCCCA
PPI-Corn-FTB GCGCCCAACACGAAATCCCATCATGCTAGAGCTGTGGCGTGATCAGCATAT-CGAGTATC-----TGACGC
DuP-Corn-FTB GCGCCCAACACGAAATCCCATCATGCTAGAGCTGTGGCGTGATCAGCATAT-CGAGTATC-----TGACGC
Pea FT-B ACCGCGGCGGAGACGCAACTCCGACGGTGAGTTCAGAGAGATCAATGGATAGTAGAATCACAGGCTTTTC
Tomato TCCCCCAACTCTCCGTCCGACCTCATAGAGATCGAACCTGACAAACACTT-CGGTTATC-----TAGGCC
Tobacco CCNCCCAATTG-----CCACTTAGAGACTTCAACAGAAAGCACTT-CGATTATC-----TCAGTTC

290 300 310 320 330 340 350
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

PPI-BnFTb -----TGGCTTTGTTACTG
eral -----TGGCTTTGTTACTG
Wiggum AAGGCTTAAGGCAGCTT---GGTCCGCGAGTTTCTTCCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG
PPI-Soy-FTB AAGGCTTAAGGCAGCTT---GGTCCGCGAGTTTCTTCCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG
DuP-Soy-FTB AAGGCTTAAGGCAGCTT---GGTCCGCGAGTTTCTTCCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG
PPI-Corn-FTB AAGGCTTAAGGCAGCTT---GGTCCGCGAGTTTCTTCCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG
DuP-Corn-FTB AAGGCTTAAGGCAGCTT---GGTCCGCGAGTTTCTTCCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG
Pea FT-B CTGGGCTGAGGCATATG---GGACCGAGCTTTTCATGTTCTAGATGCCAATCGCCCTTGGCTATGCTACTG
Tomato CTGGGCTGAGGCATATG---GGACCGAGCTTTTCATGTTCTAGATGCCAATCGCCCTTGGCTATGCTACTG
Tobacco ATATTTATCAACTCTTCGCAATATTCCTCCAAACCCCAATCTATCATTCGACCTTGGCTGTGTTACTG
AAGCTCTCAGAAACTT---GGTCCGCTGTTTCTCCCTTTTGGATGCCATCGACCATGGCTTTGCTACTG
GAGGTCTCAGAAACTT---GGTCCGCTGTTTCTCCCTTTTGGATGCCATCGACCATGGCTTTGCTACTG

360 370 380 390 400 410 420
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

PPI-BnFTb GATTCCTTCATTCAATTGCTTTGCTTGGGAGCTCTGTGGATGATGACTTAGAAAAAATGCAATCGATTTT
eral GATTCCTTCATTCAATTGCTTTGCTTGGGAGACTGTGGATGATGAATTAGAAAAAATGCAATCGATTTT
Wiggum GATTCCTTCATTCAATTGCTTTGCTTGGGAGACTGTGGATGATGAATTAGAAAAAATGCAATCGATTTT
PPI-Soy-FTB GATTCCTTCATTCAATTGCTTTGCTTGGGAGACTGTGGATGATGAATTAGAAAAAATGCAATCGATTTT
DuP-Soy-FTB GATTCCTTCATTCAATTGCTTTGCTTGGGAGACTGTGGATGATGAATTAGAAAAAATGCAATCGATTTT
PPI-Corn-FTB GATGGTTTCATCCACTTGGCTTTGCTGGATGAAGCACTTGATGATGATCTTGAGAAATGATATCATAGACTTC
DuP-Corn-FTB GATGGTTTCATCCACTTGGCTTTGCTGGATGAAGCACTTGATGATGATCTTGAGAAATGATATCATAGACTTC
Pea FT-B GATTTATTCATTCAATTGCTTTGCTGGGAGAACTCTATGATGATGATCTGGAAGATAACACTGTGCTGATTTT
Tomato GACACCTTCATTCAATCGCTTTGCTGGGAGAACTCTATGATGATGATCTGGAAGATAACACTGTGCTGATTTT
Tobacco GATACCTTCATTCAATCGCTTTGCTGGGAGAACTCTATGATGATGATCTGGAAGATAACACTGTGCTGATTTT

430 440 450 460 470 480 490
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

PPI-BnFTb CTTGGACGTTGCCAGGGCTCTGATGGTGGATATGGTGGTGGTCTGGCCAACTTCCACATCTTGGCAACA
eral CTTGGACGTTGCCAGGGCTCTGATGGTGGATATGGTGGTGGTCTGGCCAACTTCCACATCTTGGCAACA
Wiggum CTTGGACGTTGCCAGGGCTCTGATGGTGGATATGGTGGTGGTCTGGCCAACTTCCACATCTTGGCAACA
PPI-Soy-FTB CTTAACCGTTGCCAGGATCGGAATGGTGGATATGCCGGGGGAGCAGGCGAGATGCCCTCATATTGCCACAA
DuP-Soy-FTB CTTAACCGTTGCCAGGATCGGAATGGTGGATATGCCGGGGGAGCAGGCGAGATGCCCTCATATTGCCACAA
PPI-Corn-FTB TTAGCTCGATCTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGGACAGTTCGCTCATCTAGCTACGA
DuP-Corn-FTB TTAGCTCGATCTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGGACAGTTCGCTCATCTAGCTACGA
Pea FT-B CTTAACCGTTGCCAGGATCGGAATGGTGGATATGCCGGGGGAGCAGGCGAGATGCCCTCATATTGCCACAA
Tomato CTGACCCGTTGCCAGGATAAAGATGGTGGCTATGGAGGTGGACCTGGTCAGATGCCCTCATCTTGGCAACTA
Tobacco CTGAGCCGTTGCCAGGATGAAGATGGTGGCTATGGTGGTGGACCTGGTCAGATGCCCTCATCTTGGCAACTA

500 510 520 530 540 550 560
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

PPI-BnFTb GTTATGCTGCAGTGAATACACTTGTACTTTAGGAGGTGAGAAAGCCTTCTCTCAATTAACAGAGAACAA
eral GTTATGCTGCAGTGAATACACTTGTACTTTAGGAGGTGAGAAAGCCTTCTCTCAATTAATAGAGAAAA
Wiggum GTTATGCTGCAGTGAATACACTTGTACTTTAGGAGGTGAGAAAGCCTTCTCTCAATTAATAGAGAAAA
PPI-Soy-FTB CTTATGCTGCTGTAAATCACTTATTACTTTGGGTGGTGGAGAAATCCCTGGCATCAATTAATAGAGATAA
DuP-Soy-FTB CTTATGCTGCTGTAAATCACTTATTACTTTGGGTGGTGGAGAAATCCCTGGCATCAATTAATAGAGATAA
PPI-Corn-FTB CTTATGCTGCTGTAAATACACTTGTGACAAATAGGGAGCCGAAAGAGCATTGTCTATCAATCAATAGGGGCAA
DuP-Corn-FTB CTTATGCTGCTGTAAATACACTTGTGACAAATAGGGAGCCGAAAGAGCATTGTCTATCAATCAATAGGGGCAA
Pea FT-B CTTATGCTGCAGTCAATACTCTTATTACTCTGGGTGGTGGAGAAATCTTTGGCATCTATTAAATAGAAATAA
Tomato CTTATGCTGCAGTCAATCACTCAATACTTTGGGCAAACTTGAAGCTGTCTATCAATTAATAGAGAAAA
Tobacco CTTATGCTGCAGTCAATCACTCAATACTTTGGGCAAACTTGAAGCTGTCTATCAATTAATAGAGAAAA

570 580 590 600 610 620 630
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

PPI-BnFTb AATGGCTTCTTTCTTAAGACGAATGAAGGATACAAATGGAGGTTTCAGGATGCATGAATATGGGAGAAATA
eral AATGGCTTCTTTCTTAAGACGAATGAAGGATACAAATGGAGGTTTCAGGATGCATGAATATGGGAGAAATA

Wigum	AATGCTCTGTTTTTAAACACGGATGAAGGATACAAATGGAGGTTTCAGGATGCATGATATGGGAGAAATG
PPI-Soy-FTB	ACTGTATGGGTTTCTGCGCGGATGAAGCAACCAATGGTGGATTTCAGGATGCATGATGAAGGTGAAAT
DuP-Soy-FTB	ACTGTATGGGTTTCTGCGCGGATGAAGCAACCAATGGTGGATTTCAGGATGCATGATGAAGGTGAAAT
PPI-Corn-FTB	CCTGTACAATTTTATGCTGCAGATGAAGATGTATCAGGTGCTTTCAGAAATGCATGATGTTGGCGAAAT
DuP-Corn-FTB	CCTGTACAATTTTATGCTGCAGATGAAGATGTATCAGGTGCTTTCAGAAATGCATGATGTTGGCGAAAT
Pea FT-B	GTTGTACCGGTTTATGCGCGGATGAAGACGCAACGGCGGATTTCAGGATGCATGATGAGGGGAAAT
Tomato	GTTGTACACATTTTGTGCTGCGAATGAAGACGCAAGTGGTGGATTTCAGGATGCATGATGTTGGAGAAAT
Tobacco	ATTGTATACATTTTGGCTGCAAATGAAGACACAAATGGTGGCTTCAGGATGCATGATGTTGGAGAAAT
	640 650 660 670 680 690 700
PPI-BnFTb	GATGTGCGAGCTGCTACACTGCCATTTTGATTGCAAGCATCCTGAACATTGTGGATGATGAACCTACCC
eral	GATGTTTCGTGCATGCTACACTGCAATTTTCGTTGCAAGCATCCTAAAATATTATGGATGATGAACCTACCC
Wigum	GATGTTTCGTGCATGCTACACTGCAATTTTCGTTGCAAGCATCCTAAAATATTATGGATGATGAACCTACCC
PPI-Soy-FTB	GATGTTTCGAGCTTCTACACTGCCATTTTCTGTTGCAAGTGTTTGAACATTTTGGATGATGAGCTGATCC
DuP-Soy-FTB	GATGTTTCGAGCTTCTACACTGCCATTTTCTGTTGCAAGTGTTTGAACATTTTGGATGATGAGCTGATCC
PPI-Corn-FTB	GATGTTCCGTGCTTCTACACCGCTATATCGGTTGCCAGCCTTCTGAATATTCTTGATTTTAAACTGCAA
DuP-Corn-FTB	GATGTTCCGTGCTTCTACACCGCTATATCGGTTGCCAGCCTTCTGAATATTCTTGATTTTAAACTGCAA
Pea FT-B	GATGTTCCGTGCTTCTTATACTGCCATTTCTGTTGCAAGTGTCTGAAACATTTTGGATGATGAGCTGATCC
Tomato	GATGTTCCGTGCTTCTTATACTGCCATTTCTGTTGCAAGTGTATTCGAATTTGGATGATGAACCTGATTA
Tobacco	GATGTTCCGTGCTTCTTATACTGCCATTTCTGTTGCAAGTGTATTCGAATTTGGATGATGAACCTGATTA
	710 720 730 740 750 760 770
PPI-BnFTb	GCGGCTTAGGAGATTACATTTTGAGTTGCCAAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGA
eral	AGGGCTTAGGAGATTACATCTTGAGTTGCCAAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGA
Wigum	AGGGCTTAGGAGATTACATCTTGAGTTGCCAAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGA
PPI-Soy-FTB	AGAATGTTGGAGACTACATTATAAGCTGTCAAACATATGAGGGTGGCATTGCTGCTGAGCTGGTTCTGA
DuP-Soy-FTB	AGAATGTTGGAGACTACATTATAAGCTGTCAAACATATGAGGGTGGCATTGCTGCTGAGCTGGTTCTGA
PPI-Corn-FTB	AAGGTGTAGGCGACTACATAGCAAGATGTCAAACCTTATGAAGGTGGTATTGCTGGGGAGCCTTATCTGA
DuP-Corn-FTB	AAGGTGTAGGCGACTACATAGCAAGATGTCAAACCTTATGAAGGTGGTATTGCTGGGGAGCCTTATCTGA
Pea FT-B	AGAATGTTGGAGACTTCAATTTTAAAGCTGTCAAACATATGAGGGAGGCTTCTGCTGCTGAGCTGGCTCTGA
Tomato	ATGGTGTGGAAATTACATCCTAAGTTGTCAACTTATGAAGGTGGAAATTGCTGCCAACCAGGTTCTGA
Tobacco	ATGATGTTGGAAATTACATCCTAAGTTGTCAACTTATGAAGGTGGAAATTGCTGCCAACCAGGTTCTGA
	780 790 800 810 820 830 840
PPI-BnFTb	AGCTCATGGTGGGTACACCTACTGTGGGTTGGCTACTATGATTTAATCAATGAAGTCSACCGCTTGAAT
eral	AGCTCACGGTGGGTATACCTACTGTGGTTTGGCTGCTATGATTTAATCAATGAGGTCSACCGTTGAAT
Wigum	AGCTCACGGTGGGTATACCTACTGTGGTTTGGCTGCTATGATTTAATCAATGAGGTCSACCGTTGAAT
PPI-Soy-FTB	GGCTCATGGTGGGTACACCTTTTGTGGATTAGCTACAATGATTCTGATTGGTGAGGTAACTCACTTGGAT
DuP-Soy-FTB	GGCTCATGGTGGGTACACCTTTTGTGGATTAGCTACAATGATTCTGATTGGTGAGGTAACTCACTTGGAT
PPI-Corn-FTB	AGGACATGGTGGGTATACATTTCTGTGGATTGGCTGCTTGTATCTGCTTAATGAGGCGAGAAAGTTGAC
DuP-Corn-FTB	AGGACATGGTGGGTATACATTTCTGTGGATTGGCTGCTTGTATCTGCTTAATGAGGCGAGAAAGTTGAC
Pea FT-B	GGCTCATGGTGGGTATACCTTTTGTGGATTAGCTGCAATGATTCTGATTGGTGAGGTAACTCGCTTGGAT
Tomato	AGCTCATGGTGGGTATACCTTCTGTGGGTTGGCTGCAATGATTCTGATCAACGAGTAGATCGATTGGAC
Tobacco	AGCTCATGGTGGGTATACCTTCTGTGGGTTGGCTGCAATGATTCTGATTAAACGAGCGAATTCGATTGGAC
	850 860 870 880 890 900 910
PPI-BnFTb	TTGGATTCTTAATGAATTGGGTTGTATCATCGACAAGGAGTAGAAATGGGATTCAAGGTAGGACGAACA
eral	TTGGATTCTTAATGAATTGGGTTGTATCATCGACAAGGAGTAGAAATGGGATTCAAGGTAGGACGAACA
Wigum	TTGGATTCTTAATGAATTGGGTTGTATCATCGACAAGGAGTAGAAATGGGATTCAAGGTAGGACGAACA
PPI-Soy-FTB	CTGCCCTGATTAGTTGACTGGGTGGTATTCCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAACAAATA
DuP-Soy-FTB	CTGCCCTGATTAGTTGACTGGGTGGTATTCCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAACAAATA
PPI-Corn-FTB	TTGCCCTAGTTTGAATGCTGGGTGGCTTTTCCTCAAGGAGTGGAATCCGGATTTCAGGACGAACAAATA
DuP-Corn-FTB	TTGCCCTAGTTTGAATGCTGGGTGGCTTTTCCTCAAGGAGTGGAATCCGGATTTCAGGACGAACAAATA
Pea FT-B	CTGCCCTGTTTACTTGAATGGGTTGTGTTTCGCGCAAGGTAAAGAGTGTGGATTTCAGGACGAACAAATA
Tomato	TTGCCAGTTTAAATTGATTGGGTGGTATTTAGACAAGGGGTCGAAGGTGGATTTCAGGACGAACAAATA
Tobacco	TTGCCAAGATTAAATTGATTGGGTGGTATTTAGACAAGGAGTGAAGGTGGATTTCAGGACGAACAAATA
	920 930 940 950 960 970 980
PPI-BnFTb	AATTGGTCCAGGCTGCTACACGTTTTCGCGAGGAGGCCCTGTTCTACTACAGCGATTTTTTCATC
eral	AATTGGTCCAGGCTGCTACACATTTTTCGCGAGGAGGCCCTTGTGTTCTACTACAAAGATTATATCAAC
Wigum	AATTGGTCCAGGCTGCTACACATTTTTCGCGAGGAGGCCCTTGTGTTCTACTACAAAGATTATATCAAC
PPI-Soy-FTB	AATCGGTGATGGATGCTATTCCTTTTGGCAGGGAGGTGCTGTTGCTCTATTGCAAGATTATCTCTAT
DuP-Soy-FTB	AATCGGTGATGGATGCTATTCCTTTTGGCAGGGAGGTGCTGTTGCTCTATTGCAAGATTATCTCTAT
PPI-Corn-FTB	AATTGGTTGATGGTTGCTACTCCTTTTGGCAGGGAGGTGCCATTGCTTTCACACAAAAGTTAATTACGAT
DuP-Corn-FTB	AATTGGTTGATGGTTGCTACTCCTTTTGGCAGGGAGGTGCCATTGCTTTCACACAAAAGTTAATTACGAT
Pea FT-B	AATTGGTAGATGGATGCTACTCCTTTTGGCAGGGAGGTGCTGTTGCCCTATTGCAAGATTACATTCAT
Tomato	AATTAGTCGATGGCTGCTATTCCTTTTGGCAGGGAGGTGCTGTTGCTTATACAAAGATTAAATTTGAT
Tobacco	AATTAGTCGATGGCTGCTATTCCTTTTGGCAGGGAGGTGCTGTTGCTTATACAAAGATTAAATTTGAT
	990 1000 1010 1020 1030 1040 1050
PPI-BnFTb	CCAGGATATGGCACCTTCATGGATCATCATCACTATGCTCACAAGGGAGAGAT
eral	CAATGATCATGACCTTCATGGATCATCACTATGCTCACAAGGGAGAAAT

Wiggum	CAATGATCATGACCT-----TCATGGATCATCA-----CATATATCAGAAGGGACAAAT
PPI-Soy-FTB	TATCAACAAACAGATG-----GAGGAGA-CATCA-----CAGATTTTGGCGTATCTTAT
DuP-Soy-FTB	TATCAACAAACAGATG-----GAGGAGA-CATCA-----CAGATTTTGGCGTATCTTAT
PPI-Corn-FTB	TGTTGATAGCAATTGAGGTCTCTGTA-----T-----TCCTGCAAAA---GG
DuP-Corn-FTB	TGTTGATAGCAATTGAGGTCTCTGTA-----T-----TCCTGCAAAA---GG
Pea FT-B	TATCGACCAACAATG-----GCAGAGG-CATCA-----CAGTTGTTACAGTATCTGAT
Tomato	AGTCCATGAACAACCTAGGGCTGTCAAATGACCTCAGTACAGAAAGTGTGATGATTCTTCAGAGTCAGAG
Tobacco	AGTCCATGAACAACCTAGGGCTGTCAAATGACCTCAGTACAGAAAGTGTGATGATTCTTCAGAGTCAGAG

	1060	1070	1080	1090	1100	1110	1120
PPI-BnFTb	GAGGATCACCAGGA-ACATGGTCTATGATGAAGATGATCTGAAGACAGTGAAGATGA---TCTGAT						
eral	GAAGAACAT-----CATGCTCATGATGAAGATGACCTTGAAGACAGTGAAGATGATGATGATGATGATCTGAT						
Wiggum	GAGGAACAT-----CATGCTCATGATGAAGATGACCTTGAAGACAGTGAAGATGATGATGATGATGATCTGAT						
PPI-Soy-FTB	GTATCTGAAG-----CAAAAGAAAGTTTGGATGGAACCTCTAGTCA-TGCAACATGCCCTGGTGAGCAT						
DuP-Soy-FTB	GTATCTGAAG-----CAAAAGAAAGTTTGGATGGAACCTCTAGTCA-TGCAACATGCCCTGGTGAGCAT						
PPI-Corn-FTB	CCATCAGGAGAG-----GATGCCTGCAG-----CACCAGTTCATAT-----GGGTGCACC-----G-CGA						
DuP-Corn-FTB	CCATCAGGAGAG-----GATGCCTGCAG-----CACCAGTTCATAT-----GGGTGCACC-----G-CGA						
Pea FT-B	GCACCTGAAG-----AAAGGAATGTTTGGACGGAACCTCAAGTCA-TGCAACTTCCCATATTAGGCAT						
Tomato	TTATCTGATGAAGAAGAGCATTGGAGAGGATATCTCTCATGTTCA-GGATACATTTCCCTCTTGGACAA						
Tobacco	TTATCTGATGAAG-----GAGCATTGCAAGGGACATCTCTCATGTTCA-GAAGACTTGCCTCTTGGACAA						

	1130	1140	1150	1160	1170	1180	1190
PPI-BnFTb	GAGGAT-----AGCGATGAA---GATTCAGGGAAATGGTCAACCAAGTTCATCATACCTCTAC-CTAC						
eral	GAGGAC-----AACGATGAA---GATTCAGTGAATGGTCAACAGAAATCCATCATACATCCAC-CTAC						
Wiggum	GAGGAC-----AACGATGAA---GATTCAGTGAATGGTCAACAGAAATCCATCATACATCCAC-CTAC						
PPI-Soy-FTB	GAAGGC-----ACCAGTGAATTCAGTTCATCTGATTTTAAAAAATATTGCCATATAAATTTAT-TAAT						
DuP-Soy-FTB	GAAGGC-----ACCAGTGAATTCAGTTCATCTGATTTTAAAAAATATTGCCATATAAATTTAT-TAAT						
PPI-Corn-FTB	ATAACT-----CTTCTCTGCTGTGGACTATGCGAAGTTTGGATTTGATTTTATACAAC						
DuP-Corn-FTB	AAAAAT-----CTTCTCTGCTGTGGACTATGCGAAGTTTGGATTTGATTTTATACAAC						
Pea FT-B	GAAGGC-----ATGAATGAATCTGCTCATCTGACGTA---AAATATTGGTTATAAATTTAT-TAGT						
Tomato	GCAGGTGCTTGTCAGAAATGCTTCTCATAGCCCAAAATAGCAGATCTCGGATATGACTTTAT-CAAT						
Tobacco	GAAGCA-----CAGGAAATGCTTCAGATCCACAAAGATAGCAGATCTGGTTATGATTTTGT-CAAT						

	1200	1210	1220	1230	1240	1250	1260
PPI-BnFTb	ATTGACAGGAGAATTCAACTGTTTTGATAGCCTCGCCTTGCAAAGATATGTGCTCTTGTGCTCTCAGG						
eral	ATTAACAGGAGAATGCAACTGGTTTTTGTATAGCCTCGCCTTGCAAGATATGTACTCTTGTGCTCTAAGA						
Wiggum	ATTAACAGGAGAATGCAACTGGTTTTTGTATAGCCTCGCCTTGCAAGATATGTACTCTTGTGCTCTAAGA						
PPI-Soy-FTB	GAGTGGAGAGCACAAGAACCACTTTTTTACAGTATTCTTTACAGCAATATATTCTCTTATGTCACAGG						
DuP-Soy-FTB	GAGTGGAGAGCACAAGAACCACTTTTTTACAGTATTCTTTACAGCAATATATTCTCTTATGTCACAGG						
PPI-Corn-FTB	AGAGCAACCAA-ATTGGCCCACTCTTCCATTAACATTGCGCTGCAACAATACATCTACTTTGTTCTCAGG						
DuP-Corn-FTB	AGAGCAACCAA-ATTGGCCCACTCTTCCATTAACATTGCGCTGCAACAATACATCTACTTTGTTCTCAGG						
Pea FT-B	GAGTGGAGACAAAGTGAACCACTTTTTTACAGCATTGCGCTTACAGCAATATATTCTTTTATCTTACAGG						
Tomato	CGACCCATAGCTATGAGGCTCTCTTTTACAGCATGTATCTGCAAGATATGTTCTTCTTGTCTCTCAGA						
Tobacco	CGNACGATAGCTATGCGACCTGTGTTTGACAGCTTTTATCTGCAAGCAATACGTTCTTCTTGTCTCTCAGA						

	1270	1280	1290	1300	1310	1320	1330
PPI-BnFTb	TTGCTGATGGTGGATTTCAGAGACAACCTGAGGAAACCCCGTGACTTCTACCACACATGTTACTGCCTTAAG						
eral	TCGCTGACGGTGGATTTCAGAGACAACCGGAGGAAACCCCGTGACTTCTACCACACATGTTACTGCCTGAG						
Wiggum	TCGCTGACGGTGGATTTCAGAGACAACCGGAGGAAACCCCGTGACTTCTACCACACATGTTACTGCCTGAG						
PPI-Soy-FTB	AGCAAGAGGGTGGACTGAGAGACAACCGGGTAAACGTAGAGATCATTTACACACATGTTACTGTTAAG						
DuP-Soy-FTB	AGCAAGAGGGTGGACTGAGAGACAACCGGGTAAACGTAGAGATCATTTACACACATGTTACTGTTAAG						
PPI-Corn-FTB	TACTAGAGGGAGGCTTGAGCGATAAGCCTGAAAGAAACAGAGATCACTATCATTCATGCTTACTGCTCAG						
DuP-Corn-FTB	TACTAGAGGGAGGCTTGAGCGATAAGCCTGAAAGAAACAGAGATCACTATCATTCATGCTTACTGCTCAG						
Pea FT-B	AGCAAGATGGTGGCTCAGGACACAACCGGGTAAACGAGGGATCATTTATCATTCATGTTACTGTTAAG						
Tomato	TTGAAGTTGGTGGTTTCAGAGACAACCTGCGAAGGGTAGAGACTACTACCATACCTGTTACTGTTAAG						
Tobacco	T---AGATGCAGGTTTCAGAGACAACCTGCGAAGGGTAGAGACCACTACCATACCTGTTACTGTTAAG						

	1340	1350	1360	1370	1380	1390	1400
PPI-BnFTb	CGGCTTTTCGGTGGCTCAACACCGCTTGGGTCAAAAGACGAGGACACTCCTTCTTTGACTCGTGACATTTTG						
eral	CGGCTTTTCGGTGGCTCAGCACCGCTTGGTTAAAGACGAGGACACTCCTTCTTTGACTCGCGACATTTATG						
Wiggum	CGGCTTTTCGGTGGCTCAGCACCGCTTGGTTAAAGACGAGGACACTCCTTCTTTGACTCGCGACATTTATG						
PPI-Soy-FTB	TGGACTCTCATTTGTGCCAGTATAGTTGGTCAAAAGCACCAGATTCTCCACCAC-----						
DuP-Soy-FTB	TGGACTCTCATTTGTGCCAGTATAGTTGGTCAAAAGCACCAGATTCTCCACCAC-----						
PPI-Corn-FTB	TGGCCTCGCAGTTAGCCAGTACAGTGCCATGACTGATCTGCTTCTGTCGCCATTACCTCAGCATGTGCTT						
DuP-Corn-FTB	TGGCCTCGCAGTTAGCCAGTACAGTGCCATGACTGATCTGCTTCTGTCGCCATTACCTCAGCATGTGCTT						
Pea FT-B	TGGCTTGTCACTGTGCCAGTATAGTTGGTCAAAAGCAGGCGCCAGATTCTCCACCCTGCTTAAGTCAATG						
Tomato	TGGTCTTTCAATTGCTCAGTATAGCTGGACCGACGAAGCTGATTCTACACCATTACCCAGGGATGATTTT						
Tobacco	TGGTCTTTCAATTGCTCAATATAGCTGGACCAACGAAGCTGATGCGCCACCATTACCCAGGGATGATTTT						

	1410	1420	1430	1440	1450	1460	1470
PPI-BnFTb	GGTGGCTACGCA-AA--CCACCTTGAACCTGTTTCACTCTCCTCCACAACATTTGTCTTGGATGGGTATATG						
eral	GGTGGCTACTCG-AA--TCTCCTTGAACCTGTTTCACTCTTCTCACAACATTTGTCTTGGATGGGTATATG						

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Wiggum      GGTGGCTACTCG-AA--TCTCCTTGAACCTGTTCAAGTTCTTCACAAACATTGTCATGGATCAGTATAATG
PPI-Soy-FTB -----
DuP-Soy-FTB -----
PPI-Corn-FTB GGACCGTACTCT-AA--TTTGCTGGAGCCAATCCATCC-----
DuP-Corn-FTB GGACCGTACTCT-AA--TTTGCTGGAGCCAATCCATCC-----
Pea FT-B     GGCCCATACTCC-AA--TCTCTTGAACCCATCCATCCTCTCTTTAATGTTGTTTGGATCGATATCGTG
Tomato       GGTCCCTTATTCCAAATGCTGTTGGAACAGGTTCAACCACTCTTCAACGTAGTGTGGATCGGTATTATG
Tobacco      GGTCCCTTATTCTCAAAATCTTTTGAACAGATTCAACCACTTTACAAAGTAGTGTGGATCGGTATTATG

          1480      1490      1500      1510      1520      1530      1540
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
PPI-BnFTb    AAGCTTCTAGATT-----
eral         AAGCTATCGAGTCTCTTCTTTAAAGCAGCATGACCCGTTGTTGCTAATGTATGGGAAACCCCAACATAAG
Wiggum       AAGCTATCGAGTCTCTTCTTTAAAGCAGCATGACCCGTTGTTGCTAATGTATGGGAAACTCCAACATAAG
PPI-Soy-FTB -----
DuP-Soy-FTB -----
PPI-Corn-FTB -----
DuP-Corn-FTB -----
Pea FT-B     AAGCTCATGAATCTCTTTTCTCAGTTGTGACGGATGACAAGGTTTTAGCTACCAATAGCTC-GATCATTAG
Tomato       AAGCTCGCGAATACT-CTCAGGCTTGTGAGACTGTTTCAC-CACTTTCATTAGCACCAAC--TTTTTCAG
Tobacco      AAGCTCGTAGCTTCTTCTCATGCTTGTGATAATATTTTACGCGATAGCTGTAGCTGGAAT--GTTACC--

          1550      1560      1570      1580      1590      1600      1610
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
PPI-BnFTb    AGTTTCCGTAGTGTGTAACCTGTAAGATTTCAAAAG-----
eral         AGTTTTCGTAGTGTGTAACCTGTAAGATTTCAAAAGAAGTTTCACTAATTTAACCTTAAACCTGTTAC
Wiggum       AGTTTTCGTAGTGTGTAACCTGTAAGATTTCAAAAGAAGTTTCACTAATTTAACCTTAAACCTGTTAC
PPI-Soy-FTB -----
DuP-Soy-FTB -----
PPI-Corn-FTB -----
DuP-Corn-FTB -----
Pea FT-B     AATGTAAATGTAACTAAATATGAAATATGAAATACCAAAAGATATTATTGGATGAAATTCACGTGG
Tomato       AAACCTTAGTTGCAATCCAGAAGTTAAAAGTGTCATTGGGTTCAAAAGAGTTGTGATCGTTTATGTACATA
Tobacco      ---TCTAGTTG---TTCAGAATCAGAGACTAATCTATTATTTTGAGGGATTGGATTCAAAAAAAAAAAAAA

          1620      1630      1640      1650      1660      1670      1680
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
PPI-BnFTb    TTTTTTATTACGATATACCATTTATCATATCTTTGGTTTACGACTTAAAGAATTTGATGATTGTTGAAA
eral         TTTTTTATTACGATATACCATTTATCATATCTTTGGTTTACGACTTAAAGAATTTGATGATTGTTGAAA
Wiggum       TTTTTTATTACGATATACCATTTATCATATCTTTGGTTTACGACTTAAAGAATTTGATGATTGTTGAAA
PPI-Soy-FTB -----
DuP-Soy-FTB -----
PPI-Corn-FTB -----
DuP-Corn-FTB -----
Pea FT-B     ATCTAATACAACCTGCGTGGTTTTTCATTCTGATTTGATTTTATGATTTACATGAGTTAAACGTTAAACCTT
Tomato       TCCTTGCAATTTGTATACGTGATACAAGTTGAGAGAATAACGGGTACTTTCTGAACTTGCTGAACTAGCAC
Tobacco      AAAAAA-----

          1690      1700      1710      1720      1730      1740      1750
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
PPI-BnFTb    TCTTATTCATACATTTGTTAAGAGCTTAAGGCTTAATGGTTAAGCCAATGATATAAATATTTATGCAGAA
eral         TCTTATTCATACATTTGTTAAGAGCTTAAGGCTTAATGGTTAAGCCAATGATATAAATATTTATGCAGAA
Wiggum       TCTTATTCATACATTTGTTAAGAGCTTAAGGCTTAATGGTTAAGCCAATGATATAAATATTTATGCAGAA
PPI-Soy-FTB -----
DuP-Soy-FTB -----
PPI-Corn-FTB -----
DuP-Corn-FTB -----
Pea FT-B     GTAAATTCGTCTCTGGTTTAGTGAGGTCTGTAACATCAATGTGAAATTGCGAGATATGCATGTAATAGT
Tomato       GTAAATTCGTCTCTGGTTTAGTGAGGTCTGTAACATCAATGTGAAATTGCGAGATATGCATGTAATAGT
Tobacco      GTAAATTCGTCTCTGGTTTAGTGAGGTCTGTAACATCAATGTGAAATTGCGAGATATGCATGTAATAGT

          1760      1770      1780      1790      1800      1810      1820
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
PPI-BnFTb    AGCTGTTGCTTATCACCAACGGTAATATTAATAAGCAAACAAGTATTCTGTGAT-----
eral         AGCTGTTGCTTATCACCAACGGTAATATTAATAAGCAAACAAGTATTCTGTGAT-----
Wiggum       AGCTGTTGCTTATCACCAACGGTAATATTAATAAGCAAACAAGTATTCTGTGAT-----
PPI-Soy-FTB -----
DuP-Soy-FTB -----
PPI-Corn-FTB -----
DuP-Corn-FTB -----
Pea FT-B     GGCTAAGATTTACAAATCTGGATACCGGTTATTAGTGATCAGAAATTTCATTCAATTTCCCAAACGGTCA
Tomato       GGCTAAGATTTACAAATCTGGATACCGGTTATTAGTGATCAGAAATTTCATTCAATTTCCCAAACGGTCA
Tobacco      GGCTAAGATTTACAAATCTGGATACCGGTTATTAGTGATCAGAAATTTCATTCAATTTCCCAAACGGTCA

          1830      1840      1850      1860      1870      1880      1890
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
PPI-BnFTb    -----
eral         -----
Wiggum       -----
PPI-Soy-FTB -----
DuP-Soy-FTB -----
PPI-Corn-FTB -----
DuP-Corn-FTB -----
Pea FT-B     -----
Tomato       -----
Tobacco      -----

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PPI-BnFTb
eral	-----
Wiggum	-----
PPI-Soy-FTB	-----
DuP-Soy-FTB	-----
PPI-Corn-FTB	-----
DuP-Corn-FTB	-----
Pea FT-B	-----
Tomato	AAAAAAAAAA
Tobacco	-----

- 1) PPI-BnFTB; FT3 (SEQ ID NO:9)
- 2) era1 (SEQ ID NO:80)
- 3) Wiggum (SEQ ID NO:81)
- 4) PPI-Soy-FTB; FT5 (SEQ ID NO:36)
- 5) DuP-Soy-FTB (SEQ ID NO:82)
- 6) PPI-Corn-FTB; FT6 (SEQ ID NO:39)
- 7) DuP-Corn-FTB (SEQ ID NO:83)
- 8) Pea-FT-B (SEQ ID NO:84)
- 9) Tomato (SEQ ID NO:85)
- 10) Tobacco (SEQ ID NO:86)

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Tomato	DFLTRCQDKGGYGGGPGQMPLATTYAAVNSLTTLGKPEALSSINREKLYTFILRMKDASGGFRMHGG
Tobacco	DFLTRCQDEGGYGGGPGQMPLATTYAAVNSLTTLGSPKALSSINREKLYTFWLQMKDTSGGFRMHGG
	220 230 240 250 260 270 280
PPI-BnFTB	EIDVRACYTATLTLASFLNIMDELTRGPGDYILSCQTYEGGIGGEPGSEAHGGYTCGLATMILINEVDF
eral	EIDVRACYTAISVASFLNIMDELTOGLGDDYILSCQTYEGGIGGEPGSEAHGGYTCGLAAMILINEVDF
Wiggum	EMDVRACYTAISVASFLNIMDELTOGLGDDYILSCQTYEGGIGGEPGSEAHGGYTCGLAAMILINEVDF
PPI-Soy-FTB	EIDVRACYTAISVASFLNIMDELIONVGDYITSCQTYEGGIAGEPGSEAHGGYTCFGLATMILIGEVNH
DuP-Soy-FTB	EIDVRACYTAISVASFLNIMDELIONVGDYITSCQTYEGGIAGEPGSEAHGGYTCFGLATMILIGEVNH
PPI-Corn-FTB	EIDVRASYTAISVASFLNIMDELFLAKGVGDYIARQTYEGGIAGEFYAEAHGGYTCFGLAATILPNEAEK
DuP-Corn-FTB	EIDVRASYTAISVASFLNIMDELFLAKGVGDYIARQTYEGGIAGEFYAEAHGGYTCFGLAATILPNEAEK
Pea FT-B	EIDVRACYTAISVASFLNIMDELINNVGDSILSCQTYEGGIAGEPGSEAHGGYTCFGLAAMILIGEVNF
Tomato	EMDVRACYTAISVASFLNIMDELIIHGVNIIYLSCQTYEGGIAGEPGSEAHGGYTCFGLAAMILINEVDF
Tobacco	EMDVRACYTAISVASFLNIMDELINDVGNIIYLSCQTYEGGIAGEPGSEAHGGYTCFGLAAMILINEVDF
	290 300 310 320 330 340 350
PPI-BnFTB	LNLDLSLMNVVHRQGVEMGFQGRTNKLVDCGYTFWCAAPCVLLQRFSSQMAPHGSSSHMSQGTDEDHE
eral	LNLDLSLMNVVHRQGVEMGFQGRTNKLVDCGYTFWCAAPCVLLQRLYSTNDHDVHGSSSHISEGTNEEH
Wiggum	LNLDLSLMNVVHRQGVEMGFQGRTNKLVDCGYTFWCAAPCVLLQRLYSTNDHDVHGSSSHISEGTNEEH
PPI-Soy-FTB	LDLRLVWVVFROGKECGFQGRTNKLVDCGYSEWQGAVALQLRLSSINNKMEETSQIFAVSYVSEA-
DuP-Soy-FTB	LDLRLVWVVFROGKECGFQGRTNKLVDCGYSEWQGAVALQLRLSSINNKMEETSQIFAVSYVSEA-
PPI-Corn-FTB	VDLPSLIGWVAFROGVECGFQGRTNKLVDCGYSEWQGAATAFTKRLITIVDKLRSSYSCKRPSGEDACS
DuP-Corn-FTB	VDLPSLIGWVAFROGVECGFQGRTNKLVDCGYSEWQGAATAFTKRLITIVDKLRSSYSCKRPSGEDACS
Pea FT-B	LDLRLVWVVFROGKECGFQGRTNKLVDCGYSEWQGAVALQLRLHSTHDEQMAEASQFVTVSDAPEE-
Tomato	LDLRLVWVVFROGVECGFQGRTNKLVDCGYSEWQGAVALQLRLHSTHDEQMAEASQFVTVSDAPEE-
Tobacco	LDLRLVWVVFROGVECGFQGRTNKLVDCGYSEWQGAVALQLRLKSTVHEGLGLSNELSTESADDSSE
	360 370 380 390 400 410 420
PPI-BnFTB	EHGHDED-DPE--DSDEDD-S--DEDS--DEDSGNGHQVHHT-STYIDR--RMOLVEDSLGLCRYVLLCS
eral	-HAHDED-DLE--DSDDDDDS--DEDN--DEDSVNGHRHHT-STYINR--RMOLVEDSLGLCRYVLLCS
Wiggum	-HAHDED-DLE--DSDDDDDS--DEDN--DEDSVNGHRHHT-STYINR--RMOLVEDSLGLCRYVLLCS
PPI-Soy-FTB	-----KE-SLDGTSSHATCRG--EHEG--TSESSSSDFKNIAKFINEWRAQEPLEHSTALQOQYVLLCA
DuP-Soy-FTB	-----KE-SLDGTSSHATCRG--EHEG--TSESSSSDFKNIAKFINEWRAQEPLEHSTALQOQYVLLCA
PPI-Corn-FTB	-----TSSYGCTAN-----KSSSAVDYAKFGEDFIQOSNOTGPLEHNTALQOQYVLLCS
DuP-Corn-FTB	-----TSSYGCTAK-----KSSSAVDYAKFGEDFIQOSNOTGPLEHNTALQOQYVLLCS
Pea FT-B	-----KE-CLDGTSSHATSHI--RHEG--MNESSSDVKNIGNFISEWRQSEPLEHSTALQOQYVLLCS
Tomato	SELSDEEHLLEGISSHVQDTFPLGQAGACQENASHSPKADTGMEFINRPIAMRPLEDSMYLQOQYVLLCS
Tobacco	SELSDEE-HLQGTSSHVQKTCPLGEG--QENASDPTKADTGMEFINRPIAMRPLEDSMYLQOQYVLLCS
	430 440 450 460 470 480 490
PPI-BnFTB	CVADGGFRDRLRKPRDFYHTCYCLSGLSVACHAWSDDEETPPLTRDTLGGYAN-HLEFVHLHNTLVDRY
eral	KLPDGGFRDRLRKPRDFYHTCYCLSGLSVACHAWLKDEETPPLTRDTMGGYSN-LLEFVQLLHNTLVDRY
Wiggum	KLPDGGFRDRLRKPRDFYHTCYCLSGLSVACHAWLKDEETPPLTRDTMGGYSN-LLEFVQLLHNTLVDRY
PPI-Soy-FTB	CEQGGGLRDKPGKRRDHYHTCYCLSGLSLCOYSWSKHPDSPP-----
DuP-Soy-FTB	CEQGGGLRDKPGKRRDHYHTCYCLSGLSLCOYSWSKHPDSPP-----
PPI-Corn-FTB	CVLEGGGLRDKPGKRRDHYHSCYCLSGLSVQYSAMTDGSCPLPOHVTGPYSN-LLEFTH-----
DuP-Corn-FTB	CVLEGGGLRDKPGKRRDHYHSCYCLSGLSVQYSAMTDGSCPLPOHVTGPYSN-LLEFTH-----
Pea FT-B	CEQGGGLRDKPGKRRDHYHSCYCLSGLSLCOYSWSKHPDSPPPKVVMGPYSNLLLEFTHPLFNVVLDY
Tomato	CTEVGGFRDRLRKPRDFYHTCYCLSGLSVQYSWIDEADSTPLPRDVFGPYSKCLLEQVHPLFNVVLDY
Tobacco	CTED-GGFRDRLRKPRDFYHTCYCLSGLSVQYSWTNEADAPPLPRDVFGPYSONLLFQTHPLFNVVLDY
	500 510
PPI-BnFTB	YEASRE-----
eral	NEAIEEFFKAA-----
Wiggum	NEAIEEFFKAA-----
PPI-Soy-FTB	-----
DuP-Soy-FTB	-----
PPI-Corn-FTB	-----
DuP-Corn-FTB	-----
Pea FT-B	REAHEEFSQL-----
Tomato	YEAREYSQACETVSPLSLAPTFSET
Tobacco	YEAREEFSCL-----

Also included in the invention is the farnesyl transferase alpha consensus sequence of SEQ ID NO:87 and the farnesyl transferase beta consensus sequence of SEQ ID NO:88. To generate the consensus sequence, the farnesyl transferase alpha and farnesyl transferase beta sequences of the invention were aligned using the program BioEdit. The homology

between the farnesyl transferase alpha (FTA) polypeptide sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10E. The homology between the farnesyl transferase beta (FTB) polypeptide sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10F.

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Table 10E ClustalW Amino Acid Analysis of FT Alpha

		10	20	30	40	50	60	70				
10	BnA-12	MDYFRAIYFSDERSARALRL									
	At-FT-A	-----MNFDET	VPLS	QFLEWSDVVF	ET	CDGPNPVVPIA	YKEEFRET	MDYFRAIYFSDERSPRALRL				
	PPI-Soy-FTA	MESGSSEGEEOOR	VPLR	EPVLEWSDVTP	VP	CDGPNPVVPIQ	YTEEFSEV	MDYFRAIYFSDERSPRALRL				
	Consensus	-----	VPL	F	EWSDV	F	C	DGPNPVVPI	Y	EEF	E	MDYFRAIYFSDERSPRALRL
		80	90	100	110	120	130	140				
15	BnA-12	TTEALRLNSGNYTVWHFGRVLVLELNNDLYEELKFIESIAEDNSKNYQL----WHHRRWVAEKLGPDVAG									
	At-FT-A	TEETLLNSGNYTVWHFRRLVLEALNNDLEEFLEFIERIAEDNSKNYQL----WHHRRWVAEKLGPDVAG										
	PPI-Soy-FTA	TAEAVQNSGNYTVWHFRRLLELSIKVDLNDLEEFVERMAAGNSKNYQMXMFCRHFRRWVAEKLGP	EARN									
	Consensus	TEEARL	LNSGNYTVWHFRRLVLE	LN	DL	EELEFIERIAEDNSKNYQL	----	WHHRRWVAEKLGPDVAG				
20		150	160	170	180	190	200	210				
25	BnA-12	LEKEFTRRVLSLDAKHYYHAWSHRQWALQALGGWENELNYCHELLEADVFNNSAWNORYYYVITRSPSLGGL									
	At-FT-A	RELEFTRRRVLSLDAKHYYHAWSHRQWTLRALGGWEDELNYCHELLEADVFNNSAWNORYYYVITQSPLLGGL										
	PPI-Soy-FTA	NELEFTKKKLSYDAKHYYHAWSHRQWALCTLGWEDELNYCTELLKEDEIFNNNSAWNORYYEVITRSPFLGGL										
	Consensus	ELEFTRRRVLSLDAKHYYHAWSHRQWALQALGGWEDELNYCHELLEADVFNNSAWNORYYYVITRSE	LGGL									
		220	230	240	250	260	270	280				
30	BnA-12	EAMRESEVSYTYKAILANPGENESSWRYLKALYKDDTESWISDPVSSVCLKVLSPADCFHGFALSTLLDL									
	At-FT-A	EAMRESEVSYTIKAILTNPANESSWRYLKALYKDDKESWISDPVSSVCLNVLSTRDCFHGFALSTLLDL										
	PPI-Soy-FTA	KAMRESEVLYTIEAIPAYPENESSWRYLRGLYKGETTSWVNDSPVSSVCLKHLRTKSNXYFALSTLLDL										
	Consensus	EAMRESEVSYTIKAILANP	NESSWRYLKALYKDDTESWISDPVSSVCLKVLSPD	CFHGFALSTLLDL								
35		290	300	310	320	330	340	350				
	BnA-12	LCDGLRPTNEHRDSVKALAN-----EEPETNLANLVCTILCRVDPIRANYWAWKL									
	At-FT-A	LCDGLRPTNEHKDSVRALAN-----EEPETNLANLVCTILCRVDPIRANYWAWRKSKITVA										
	PPI-Soy-FTA	ICFCYQENEDIRDAIDAIKTADMDKQDLDDDEKGEQNLNIA	RNTCSILKQVDPIRTNYWIRKSR	EP								
	Consensus	LCDGLRPTNEHRDSV	ALAN	----	EEPETNLANLVCTIL	CRVDPIRANYWAWRKS						
40		35										
	BnA-12	--	(SEQ ID NO:7)									
	At-FT-A	AI	(SEQ ID NO:2)									
	PPI-Soy-FTA	--	(SEQ ID NO:33)									
	Consensus	--	(SEQ ID NO:87)									
45												

Table 10F ClustalW Amino Acid Analysis of FT Beta

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		220	230	240	250	260	270	280
5	PPI-BnFTB	GE	PG	SE	AH	GG	Y	T
	PPI-Soy-FTB	GE	PG	SE	AH	GG	Y	T
	PPI-Corn-FTB	GE	PG	SE	AH	GG	Y	T
	Consensus	GE	PG	SE	AH	GG	Y	T
10	PPI-BnFTB	Q	R	F	S	S	O	M
	PPI-Soy-FTB	Q	R	L	S	S	I	N
	PPI-Corn-FTB	Q	R	L	S	S	I	N
	Consensus	Q	R	L	S	S	I	N
15	PPI-BnFTB	Q	R	L	S	S	I	N
	PPI-Soy-FTB	Q	R	L	S	S	I	N
	PPI-Corn-FTB	Q	R	L	S	S	I	N
	Consensus	Q	R	L	S	S	I	N
20	PPI-BnFTB	T	G	P	F	H	S	I
	PPI-Soy-FTB	T	G	P	F	H	S	I
	PPI-Corn-FTB	T	G	P	F	H	S	I
	Consensus	T	G	P	F	H	S	I
25	PPI-BnFTB	N	H	L	E	P	F	H
	PPI-Soy-FTB	N	H	L	E	P	F	H
	PPI-Corn-FTB	N	H	L	E	P	F	H
	Consensus	N	H	L	E	P	F	H

Also included in the invention is the farnesyl transferase alpha consensus sequence of SEQ ID NO:89 and the farnesyl transferase beta consensus sequence of SEQ ID NO:90. To generate the consensus sequence, the farnesyl transferase alpha and farnesyl transferase beta sequences of the invention were aligned using the program BioEdit. The homology between the farnesyl transferase alpha (FTA) nucleic acid sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10G. The homology between the farnesyl transferase beta (FTB) nucleic acid sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10H.

Table 10G ClustalW Nucleic Acid Analysis of FT Alpha

		10	20	30	40	50	60
40	BnA-12	-----	-----	-----	-----	-----	1
	At-FT-A	-----	GAGT	CGGG	GAAC	ATGA	ATTC
	PPI-Soy-FTA	ATGG	AATCTGGG	TCTAG	CGGA	ACGAGA	AGAGGT
	Consensus	-----	CG	GA	GA	GA	GA
45	BnA-12	-----	-----	-----	-----	-----	1
	At-FT-A	AT	TGGAGTGGTC	CAGAC	CGTGGT	CCAT	TGACTCAGG
	PPI-Soy-FTA	AGTGG	AGTGGTCAGAT	GTGT	TACTCCGG	TCTCTCA	AAACGAC
	Consensus	A	TGGAGTGGTCAG	GT	CC	T	CTCA
50	BnA-12	-----	-----	-----	-----	-----	1
	At-FT-A	AT	TGGAGTGGTC	CAGAC	CGTGGT	CCAT	TGACTCAGG
	PPI-Soy-FTA	AGTGG	AGTGGTCAGAT	GTGT	TACTCCGG	TCTCTCA	AAACGAC
	Consensus	A	TGGAGTGGTCAG	GT	CC	T	CTCA
55	BnA-12	-----	-----	-----	-----	-----	1
	At-FT-A	AAT	TGCC	TACAAG	GAAGAT	TTC	CGGAG
	PPI-Soy-FTA	GAT	CCAGTACA	CTGAAG	AGTTTC	CGGAG	TATGG
	Consensus	AT	TACA	GAAGAT	TTC	CGA	TATGG
60	BnA-12	-----	-----	-----	-----	-----	1
	At-FT-A	CGAC	GAGCG	TCTG	CTCGCG	CTG	GCACT
	PPI-Soy-FTA	CGAT	GAGCG	TCTG	CTCGCG	CTG	GCACT
	Consensus	CGAC	GAGCG	TCTG	CTCGCG	CTG	GCACT
65	BnA-12	-----	-----	-----	-----	-----	1
	At-FT-A	CGAC	GAGCG	TCTG	CTCGCG	CTG	GCACT
	PPI-Soy-FTA	CGAT	GAGCG	TCTG	CTCGCG	CTG	GCACT
	Consensus	CGAC	GAGCG	TCTG	CTCGCG	CTG	GCACT

5	BnA-12	CAACTACAGCGTGTGGCATTTCGGGCGCTTAGTACTCGAGGAGCTTAATAACGACTTGTG	149
	At-FT-A	CAACTACACAGTGTGGCATTTCAGGCGCTTAGTACTCGAGGCGCTTAATCAGGACTTGTG	287
	PPI-Soy-FTA	CAACTACACTGTGTGGCATTTCACCGCTTGTACTTCAGTTCGCTAAAGTTCGACTTGA	299
	Consensus	CAACTACAGTGTGGCATTTCGGGCGCTTAGTACTCGAGGCGCTTAATACGACTTGTG	224
10	BnA-12	TGAAGAGCTCAAGTTCATCGAAAGCATTGCTGAGGATAACTCTAAGAAGTACCAGTTGTG	209
	At-FT-A	TGAAGAAGTCGAGTTCATCGAACGCATTGCTGAGGATAACTCTAAGAAGTACCAGTGTG	347
	PPI-Soy-FTA	CGATGAAGTTCGAGTTCGAGCGGTATCGCCCTGGAATTTCTAAAAATATCAGATGTG	359
	Consensus	TGAAGAAGTCGAGTTCATCGAACGCATTGCTGAGGATAACTCTAAGAAGTACCAGTGTG	283
15	BnA-12	G-----CATCATCGACGATGGGTCCAGAGAAACTGGGTCCTGATGTTGCAGG	257
	At-FT-A	G-----CATCATCGCGATGGGTTGCAGAGAAACTGGGTCCTGATGTTGCAGG	395
	PPI-Soy-FTA	NATGTTCTGTAGGCATCTAGACGATGGGTTGCCGAGAAATTAGGTCCTGAAGCTAGAAA	419
	Consensus	G-----CATCATCGACGATGGGTTGCAGAGAAACTGGGTCCTGATGTTGCAGG	331
20	BnA-12	AAAGCAACTTGAGTTTACTCCGAGGGTACTATCACTTGATGCCAAGCATTATCATGCTTG	317
	At-FT-A	GAAGCAACTTGAAATTTACCCGTAGCTACTTTCAGTTGATGCCAAACATTATCATGCTTG	455
	PPI-Soy-FTA	CAATGAGCTCGAGTTCACCAAAAGATACTGTCGTTGATGCCAAACATTATCATGCTTG	479
	Consensus	AAAGCAACTTGAGTTTACCCGAGGGTACTATCACTTGATGCCAAACATTATCATGCTTG	387
25	BnA-12	GTACACATAGGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAGATGAGCTTAACTACTG	377
	At-FT-A	GTACACATAGGCAGTGGGACACTACGGGCATTAGGAGGATGGGAAGATGAGCTCGATTACTG	515
	PPI-Soy-FTA	GTCTCATAGACAGTGGGCTCTTCAACACCTAGGAGGATGGGAAGATGAACTTAATTATTG	539
	Consensus	GTACACATAGGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAGATGAGCTTAACTACTG	446
30	BnA-12	CCACGAGCTCCTTGAAGCTGACGCTCTTAACAACCTCTGCATGGAATCAGAGGTATTACGT	437
	At-FT-A	TCACGAGCTCCTTGAAGCTGACGCTCTTAACAATTCGCTCGGAATCAGAGGTATTATGT	575
	PPI-Soy-FTA	CACAGAACTACTTAAGAAGACATTTTAACAATTCTGCTTGGAAATCAGAGATATTTTGT	599
	Consensus	CCACGAGCTCCTTGAAGCTGACGCTCTTAACAATTCGCTTGGAAATCAGAGGTATTATGT	505
35	BnA-12	TATAACTAGATCAACCTTCGTTGGGAGGCCTAGAAGCCATGAGAGAATCTGAAGTAAGCTA	497
	At-FT-A	CATCAACCAATCTCCTTTGTTGGGAGGCCTAGAAGCCATGAGAGAATCTGAAGTAAGCTA	635
	PPI-Soy-FTA	CATAACAAGCTCTCCTTTCTGGGGGGCCTAAAGCTATGAGAGAGTCTGAAGTCTTTTA	659
	Consensus	CATAACAGATCTCCTTTGTTGGGAGGCCTAGAAGCCATGAGAGAATCTGAAGTAAGCTA	564
40	BnA-12	CACAGTCAAGCCATTTTAGCAAAATCCGGGAACGAGAGCTCTGGAGCTACCTCAAAGC	557
	At-FT-A	CACAATCAAGCCATTTTAACCAATCTGCAACGAGAGCTCATGGCGATACCTAAAAGC	695
	PPI-Soy-FTA	CACCATCGAAGCCATTATAGCCCTACCTGAAATGAAAGCTCTGGAGATATCTACGAGG	719
	Consensus	CACAATCAAGCCATTTTAGCCAATCTCTGAACGAGAGCTCTGGAGATACCTAAAAGC	622
45	BnA-12	CCTTTACAAAGACGACACAGAGTCTTGGATTAGTGATCCAAGTGTTCCTCAGTCTGTTT	617
	At-FT-A	GCTTTACAAAGACGACAAAGAAATCCTGGATTAGTGATCCAAGTGTTCCTCAGTCTGTTT	755
	PPI-Soy-FTA	ACTTTATAAAGGTGAAGTACTTCAATGGGTAAATGATCCTCAAGTTCCTTCAGTATGCTT	779
	Consensus	CCTTTACAAAGACGACACAGAATCTTGGATTAGTGATCCAAGTGTTCCTCAGTCTGTTT	679
50	BnA-12	GAAAGTTCTCTCACGCGCGGACTGCTTCCATGGATTGCTCTGAGCACCCCTTTTGGATCT	677
	At-FT-A	GAATGTTCTATCCCGCACAGATTGCTTCCATGGATTGCTCTGAGCACCCCTTTTGGATCT	815
	PPI-Soy-FTA	AAAGATTTTGA---CAACTAAGAGCAACTACCTGTTTGCTCTTAGCACATTTTATGATCT	836
	Consensus	GAAATTTCTCTCACGCGCGGACTGCTTCCATGGATTGCTCTGAGCACCCCTTTTGGATCT	734
55	BnA-12	TCCTGCGATGGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	737
	At-FT-A	TCATATGATGGACTGAGACCAACCAACGAGCATAAAGACTCAGTGAGCTCTAGCTAA	875
	PPI-Soy-FTA	TATATGCTTTGGTTATCAACCAAAATGAAGACATTAGAGATGCAATGACGCTTAAAGAC	896
	Consensus	TCATATGCGATGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	792
60	BnA-12	TCCTGCGATGGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	737
	At-FT-A	TCATATGATGGACTGAGACCAACCAACGAGCATAAAGACTCAGTGAGCTCTAGCTAA	875
	PPI-Soy-FTA	TATATGCTTTGGTTATCAACCAAAATGAAGACATTAGAGATGCAATGACGCTTAAAGAC	896
	Consensus	TCATATGCGATGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	792
65	BnA-12	TCCTGCGATGGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	737
	At-FT-A	TCATATGATGGACTGAGACCAACCAACGAGCATAAAGACTCAGTGAGCTCTAGCTAA	875
	PPI-Soy-FTA	TATATGCTTTGGTTATCAACCAAAATGAAGACATTAGAGATGCAATGACGCTTAAAGAC	896
	Consensus	TCATATGCGATGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	792
70	BnA-12	TCCTGCGATGGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	737
	At-FT-A	TCATATGATGGACTGAGACCAACCAACGAGCATAAAGACTCAGTGAGCTCTAGCTAA	875
	PPI-Soy-FTA	TATATGCTTTGGTTATCAACCAAAATGAAGACATTAGAGATGCAATGACGCTTAAAGAC	896
	Consensus	TCATATGCGATGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	792
75	BnA-12	TCCTGCGATGGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	737
	At-FT-A	TCATATGATGGACTGAGACCAACCAACGAGCATAAAGACTCAGTGAGCTCTAGCTAA	875
	PPI-Soy-FTA	TATATGCTTTGGTTATCAACCAAAATGAAGACATTAGAGATGCAATGACGCTTAAAGAC	896
	Consensus	TCATATGCGATGGTTGAGACCAACCAACGAGCATAGAGACTCGTGAAAGCTCTAGCTAA	792

5	BnA-12	TGAAGAACCAGAGACTAACTTGGCCAATTGGTGTGTACATTCTGTGTCGTGTGATCC	797
	At-FT-A	TGAAGAACCAGAGACTAACTTGGCCAATTGGTGTGTACTATTCTTGGTCGTGTAGATCC	935
	PPI-Soy-FTB	CGCAGAA--TATGGATAAACAACATTATGATGATGATGAGAAAGGGGAACAACAAATTTA	954
	Consensus	TGAAGAACCAGAGACTAACTTGGCCAATTGGTGTGTACATTCTGTGTCGTGTAGATCC	850
10	BnA-12	970 980 990 1000 1010 1020 AATA-AGAGCTAACTATTGGGC--ATGG-----	822
	At-FT-A	TATA-AGAGCTAACTATTGGGC--ATGGAGGAAGAGCAAGATTACAGTGGCAGCAATTTG	992
	PPI-Soy-FTB	AATATAGCAGCAAAATATTTCTCTATCCTAAACAAGTTGATCCAATTAGAACCACATAT	1014
	Consensus	AATA-AGAGCTAACTATTGGGC--ATGGAAAGAGCAAGATTACAGTGGCAGCAATTTG	889
15	BnA-12	1030 1040 1050 1060 1070 1080 AATA-AGAGCTAACTATTGGGC--ATGG-----	822
	At-FT-A	AATATGTGACGCCCCCAAAATCACAATTGAAAAAGACTTGATTATTAGTTTTTACGTAATT	1052
	PPI-Soy-FTB	TGGATTGTGCGCAAGAGCGACTTCTCT-----	1041
	Consensus	ATGTGCGCAAGAGCGACTTCTCT-----	900
20	BnA-12	1090 1100 1110 1120 1130 1140 AATA-AGAGCTAACTATTGGGC--ATGG-----	822
	At-FT-A	AACTGCTTATTTATGAATCAGATGTTTCATGTTAACATGTATCAAAACAATCTTGATTCT	1112
	PPI-Soy-FTB	-----	1041
	Consensus	-----	900
25	BnA-12	1150 1160 1170 AATA-AGAGCTAACTATTGGGC--ATGG-----	822
	At-FT-A	CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1143
	PPI-Soy-FTB	-----	1041
	Consensus	-----	900
30	BnA-12	822 (SEQ ID NO:6)	
	At-FT-A	1143 (SEQ ID NO:1)	
	PPI-Soy-FTB	1041 (SEQ ID NO:31)	
	Consensus	900 (SEQ ID NO:89)	

Table 10H ClustalW Nucleic Acid Analysis of FT Beta

35	PPI-BnFTb	10 20 30 40 50 60 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	GGCGGATCCCGACCTACCGAGGCTCACGGTGACGCAGGTGGAGCAGATGAAGGTGGAGGC	60
40	PPI-BnFTb	70 80 90 100 110 120 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----GCCACCATTCTCGCAACGCCCAACCCCTCAT	32
	Consensus	CAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCGCGCCCAACAGAAATCCATCAT	120
45	PPI-BnFTb	130 140 150 160 170 180 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	-----	1
50	PPI-BnFTb	190 200 210 220 230 240 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	-----	1
55	PPI-BnFTb	250 260 270 280 290 300 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	-----	1
60	PPI-BnFTb	310 320 330 340 350 360 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	-----	1
65	PPI-BnFTb	370 380 390 400 410 420 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	-----	1
70	PPI-BnFTb	430 440 450 460 470 480 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	-----	1
75	PPI-BnFTb	490 500 510 520 530 540 -----	1
	eral	-----	1
	PPI-Soy-FTB	-----	1
	Consensus	-----	1

5	eral	TGGACGCTGCCAGGCTCTGAAGGTGGATACGGTGGTGGTCTGGGCCAAGTTCCACATCT	239
	PPI-Soy-FTB	TAACCGTTGCCAGGATCCGAATGGTGGATATCGCGGGGAGCCAGGCCAGATGCCCATAT	272
	PPI-Corn-FTB	AGCTCGATGTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGGACAGTGCCTCACCT	360
	Consensus	TG CG GCCAGG T C GATGGTGGATATGGTGGTGG CCTGGCCA T CC CATCT	160
		370 380 390 400 410 420	
10	PPI-BnFTb	TGCAACAGTTATGCTGCAGTGAATCACTTGTACTTTAGGAGGTGAGAAAGCCTTCTC	206
	eral	TGCAACTACTTATGCTGCAGTGAATCACTTGTACTTTAGGAGGTGACAAAGCCCTTTC	299
	PPI-Soy-FTB	TGCCACAACCTATGCTGCTGTTAATTCACCTTATTACTTTGGTSGTGAGAAATCCCTGGC	332
	PPI-Corn-FTB	AGCTAGCACTTATGCTGCTGTAATACACTTGTACAAATAGGGAGCGAAAAGCATTTGTC	420
	Consensus	TGC AC ACTTATGCTGC GT AAT CACTTGTACTTTAGG GGTGA AAAGCC T TC	211
15		430 440 450 460 470 480	
	PPI-BnFTb	TTCAATTAACAGAGAACAAATGGCTTGTCTTAAACAGCAATGAAGGATACAAATGGAGG	266
	eral	TTCAATTAATAGAGAAAAATGCTTGTCTTTTAAAGACGGATGAAGGATACAAATGGAGG	359
	PPI-Soy-FTB	ATCAATTAATAGAGATAAACTGTATGGCTTTCTGCGCGGGATGAAGCAACCAATGGTGG	392
	PPI-Corn-FTB	ATCAATCAATAGGGCAACCTGTACAATTTATGCTGACAGATGAAGATGTATCAGGTGC	480
	Consensus	TCAATTAATAGAGA AAA TGT T GTTTT T G CGGATGAAGGAT CAA TGC GC	259
20		490 500 510 520 530 540	
	PPI-BnFTb	TTTCAGGATGCATAATATGGGACAAATAGATGTCGGAGCCTGCTACACTGCCATTTCAT	326
	eral	TTTCAGGATGCATGATATGGGACAAATAGATGTCGGTGCATGCTACACTGCCAATTCGGT	419
	PPI-Soy-FTB	ATTTCAGGATGCATGATGAAGGTGAAATTGATGTTCCGAGCTTCTACACTGCCATTCTGT	452
	PPI-Corn-FTB	TTTCAGAAATGCATGATGGTGGCGAAATTGATGTCGGTGCCTTCTACACCGCTATACGGT	540
	Consensus	TTTCAGGATGCATGAT GG GAAATTGATGT CG GC TGCTACACTGC ATTCGGT	311
25		550 560 570 580 590 600	
	PPI-BnFTb	TGCAAGCATCCTGAACATTGTGGATGATGAACACACCCGCGGCTTAGGAGATTACATTTT	386
	eral	TGCAAGCATCCTGAATATTATGGATGATGAACACACCCAGGGCTTAGGAGATTACATCTT	479
	PPI-Soy-FTB	TGCAAGTGTTTTGAAACATTTTGGATGATGAGCTGATCCAGAATGTGGAGACTACATAT	512
	PPI-Corn-FTB	TGCCAGCCTTGTGAATATTCTTGATTTTAACTGGCAAAAGGTGTAGGCGACTACATAGC	600
	Consensus	TGCAAGC T TGAA ATT TGGATGATGAAC ACCCA GG TAGGAGA TACAT T	359
30		610 620 630 640 650 660	
	PPI-BnFTb	GAGTTGCCAAACTTATGAAGGTGGCATTGAGGGGAACCTGGCTCCGAAGCTCATGTTGG	446
	eral	GAGTTGCCAAACTTATGAAGGTGGCATTGAGGGGAACCTGGCTCCGAAGCTCACGGTGG	539
	PPI-Soy-FTB	AAGCTGTCAAACATATGAGGGTGGCATTGCTGTTGAGCCTGGTTCTGAGGCTCATGTTGG	572
	PPI-Corn-FTB	AAGATGTCAAACCTTATGAAGGTGGTATTGCTGGGGAGCCTTATGCTGAAGCACATGGTGG	660
	Consensus	AG TG CAAACTTATGAAGGTGGCATTG GGGGA CCTGG TC GAAGCTCATGTTGG	411
35		670 680 690 700 710 720	
	PPI-BnFTb	GTACACGTACTGTGGGTTGGCTACTATGATTTAATCAATGAAGTCGACCGCTTGAATTT	506
	eral	GTATACCTACTGTGGTTTGGCTGCTATGATTTAATCAATGAGGTCGACCGTTTGAATTT	599
	PPI-Soy-FTB	GTACACCTTTTGTGGATTAGCTACAATGATTCTGATTGGTGAGGTTAATCACTTGGATCT	632
	PPI-Corn-FTB	GTATACATTCTGTGGATTGGCTGCTTTGATCCTGCTTAATGAGGCAGAGAAAGTTGACTT	720
	Consensus	GTA AC T CTGTGG TTGGCT CTATGATT T AT AATGAGGT GA C TTG ATTT	458
40		730 740 750 760 770 780	
	PPI-BnFTb	GGATTTCGTTAATGAATTGGGTTGTACATCGACAAGGAGTAGAAATGGGATTCCAAGGTAG	566
	eral	GGATTTCATTAAATGAATTGGGCTGTACATCGACAAGGAGTAGAAATGGGATTCCAAGGTAG	659
	PPI-Soy-FTB	GCCTCGATTACTTGACTGGGTGCTATTCCGACAAGGTAAGGAATGTGGATTCCAAGGGAG	692
	PPI-Corn-FTB	GCCTAGTTTGTATGGCTGGGTGCTTTTCGTCAAGGAGTGAATGCGGATTCCAAGCACG	780
	Consensus	C T TTAAT A TGGGT GTA TCGACAAGGAGT GAA GGATT CAAGG AG	501
45		790 800 810 820 830 840	
	PPI-BnFTb	GACGAACAAATPGGTGCACGGTTGCTACACGTTTTGGCAGCCAGCCCTGCTTCTACT	626
	eral	GACGAACAAATPGGTGCATGGTTGCTACACATTTTGGCAGGCAGCCCTTGTGTTCTACT	719
	PPI-Soy-FTB	AACAATAAACTGGTGATGGATGCTATTCTTTTGGCAGGGAGGTGCTGTGCTCTATT	752
	PPI-Corn-FTB	AACTAATAAATPGGTGATGGTTGCTACTCCTTTTGGCAGGGAGGTGCCATTGCTTTTAC	840
	Consensus	AC AA AAATPGGT GATGGTTGCTAC C TTTTGGCAGG AGC C TG TCTA T	547
50		850 860 870 880 890 900	
	PPI-BnFTb	ACAGCGATTTTTTCATCCCAGGATATGGCACCTCATGATCATCATCATATATGCAC	685
	eral	ACAAAGATTATATTCACCAATGATCATGACGTTCATGATCATCA---CATATATCAG	775
	PPI-Soy-FTB	GCAAAGATTATCTTCTATTATCAAC-AAACAGATGGAAAGACATCA-C-----AGATTT	805
	PPI-Corn-FTB	ACAAAGTTAATTACGATTGTTGAT-AAGCAA-----	871
	Consensus	ACAAAGATTAT TTC A GAT-A G A G CATCA - -	574
55		910 920 930 940 950 960	
	PPI-BnFTb	ACAGCGATTTTTTCATCCCAGGATATGGCACCTCATGATCATCATCATATATGCAC	685
	eral	ACAAAGATTATATTCACCAATGATCATGACGTTCATGATCATCA---CATATATCAG	775
	PPI-Soy-FTB	GCAAAGATTATCTTCTATTATCAAC-AAACAGATGGAAAGACATCA-C-----AGATTT	805
	PPI-Corn-FTB	ACAAAGTTAATTACGATTGTTGAT-AAGCAA-----	871
	Consensus	ACAAAGATTAT TTC A GAT-A G A G CATCA - -	574

5	PPI-BnFTb	AAGGGACGATGAAGATCAGGAGGACATGGTCATGATGAAGATGATCCTGAAGACAGTG	745
	eral	AAGGGACAAATGAAGAAAT-----CATGCTCATGATGAAGATGACCTTGAAGACAGTG	829
	PPI-Soy-FTB	TTGCGGTATCTTATCTATCTGAAGCAAAAGTTGGATCGAACCTCTAGTCATGCAA	865
	PPI-Corn-FTB	TTGAGGT-CCTCGTATTCTG-----CAAAAGCCCATCGGAGAGGATGCCTG--CA-GCAC	924
	Consensus	G G A T A G C T G A A G C A T A G A G C C T G A	598
10	PPI-BnFTb	ATCAAGATGAT---TCTGATGAGGATAGCGATGAAGATTCAGGGAATGGTCACCAAGTTC	802
	eral	ATGATGATGATGATTCTGATGAGGACCAACGATGAAGATTCAGTGAATGGTCACCAAGTTC	889
	PPI-Soy-FTB	CATGCCGTGGTGAGCATGAAGGACCAAGTGAATCCAGTTCATCTGATTTTAAATATATG	925
	PPI-Corn-FTB	CAGTTCATA-TGGGTGCACCGCAATAAGTCTTCTCTGCTCTGGACTATGCCAAGTTTG	983
	Consensus	G ATG TG T TGA G G A A G A T T T C A G G A T T A A A T T	629
20	PPI-BnFTb	ATCATACTCTACCTACATTGACAGGAGAAATCAACCTGTTTTTGATAGCCTCGGCTTGC	862
	eral	ATCATACTACCTACCTACATTAAACAGGAGAAATGCAACTGGTTTTTGATAGCCTCGGCTTGC	949
	PPI-Soy-FTB	CCTATAAATTTTAAATGAGTGGAGAGCAAGAAGCACTTTTTCAGTATTCCTTTAC	985
	PPI-Corn-FTB	GATTTGATTTTATACAAACAGAGCAACCAAAATGGCCCACTCTTCCATAACATTCCTTGC	1043
	Consensus	A T A T T A A C A G A A A T A A C C T T T T A T A G C T T G C T T G C	663
30	PPI-BnFTb	AAAGATATGTGCTCTTGCTCTCTCAGGTGCTGATGGTGGATTGAGAGACAAGCTGAGGA	922
	eral	AGAGATATGTACTCTTGCTCTTAAATCTGACGGTGGATTGAGAGACAAGCCGAGGA	1009
	PPI-Soy-FTB	AGCAATATATTTCTTATCTGACAGGAGCAAGAGGGTGGATGAGAGACAACCGGGA	1045
	PPI-Corn-FTB	AACAATACATCTTCTTCTCTCAGGTACTAGAGGGAGGCTTGAGGATTAAGCCTGGAA	1103
	Consensus	A A T A T T C T C T T T G T C T C A G G T C G A G G T G G A T T A G A G A C A A G C C G G A	709
35	PPI-BnFTb	AACCCCTGACTTCTACACACATGTTACTGCCTAAGCGGTCTTTCCGTGGCTCAACAG	982
	eral	AACCCCTGACTTCTACACACATGTTACTGCCTGAGCGGTCTTGTCTGTGGCTCAGCAG	1069
	PPI-Soy-FTB	AACGTACAGATCATTATCACACATGTTACTGTTTAACTGGATCTCATTTGTGCCAGTATA	1105
	PPI-Corn-FTB	AGAACAGAGATCACTATCATGCTACTGCCTCAGTGGCTCGCAGTTAGCCAGTACA	1163
	Consensus	A A C C G G A C T A C A C A C A T G T T A C T G C C T A G C G G T C T T C C G T G G C T C A A C A G	752
45	PPI-BnFTb	CTTGGTCAAAAGAGGAGGACACTCCCTCTTGAATCTGTGACATTTTGGGTGGCTACGCCAA	1042
	eral	CTTGGTTAAAAGAGGAGGACACTCCCTCTTGAATCTGTGACATTTTGGGTGGCTACTCGA	1129
	PPI-Soy-FTB	GTGGTCAAAAGCAGCCAGATTCTCCACCAC-----	1135
	PPI-Corn-FTB	GTGCCATGACTGATACTGTTCTGTCGCCAATTACCTCAGCATGTGCTTGCACCGTACTCTA	1223
	Consensus	T T G G T A A A G A C G A C T C C C T T G A A T C T G T G A C A T T T G G T G G C T A C G C C A A	786
50	PPI-BnFTb	ACCACCTTGAACCTGTTCACTCTCCACAAACATTGTCTTGGATCGGTATTATGAAGCTT	1102
	eral	ATCTCCTTGAACCTGTTCACTCTCCACAAACATTGTCTTGGATCGGTATTATGAAGCTT	1189
	PPI-Soy-FTB	ATCTCCTTGAACCTGTTCACTCTCCACAAACATTGTCTTGGATCGGTATTATGAAGCTT	1135
	PPI-Corn-FTB	ATTTGCTCGAGCCAATCCATCC-----	1245
	Consensus	A C T G A C C T T G A A C C T G T T C A C T C T C C A C A A C A T T G T C T T G G A T C G G T A T T A T G A A G C T T	797
60	PPI-BnFTb	CTAGATTT-----	1110
	eral	TCGAGTTCTTCTTTAAAGCAGCATGACCCGTGTTGCTAATGTATGGGAAACCCCAACA	1249
	PPI-Soy-FTB	-----	1135
	PPI-Corn-FTB	-----	1245
	Consensus	-----	797
70	PPI-BnFTb	-----	1110 (SEQ ID NO:8)
	eral	TAAGAGTTTCCGTAGTGTGTAACCTGTAAGATTTCAAAG	1290 (SEQ ID NO:73)
	PPI-Soy-FTB	-----	1135 (SEQ ID NO:34)
	PPI-Corn-FTB	-----	1245 (SEQ ID NO:37)
	Consensus	-----	797 (SEQ ID NO:90)

Example 5: Vector constructs for Transformation

The FTA or FTB sequences have been used to produce constructs suitable for transformation into plants and under the control of appropriate regulatory sequences. The gene sequences were in either the sense orientation for over-expression or the antisense orientation for down-regulation. Portions of these sequences have been used to construct a double-stranded-RNA-inhibition (dsRNAi) construct. A sequence of preferably not less than 21 nt was cloned as an inverse repeat separated by a linker that when expressed results in down-regulation of the target gene. Double antisense (DA) vectors have been created in which a direct repeat of an antisense sequence is separated by a spacer sequence such as GUS. Promoters have been used for constitutive expression such as the 35S CaMV promoter, the MuA *Zea mays* promoter or inducible by specific environmental or cellular cues such as the ABA levels or drought conditions which induce expression of the RD29A promoter. Alternatively, tissue or organelle specific promoters such as the HIC or CUT1 promoter can be used. Such constructs have been transformed into *Arabidopsis thaliana*, *Brassica*, *Zea mays*, *Glycine max*. Other species can be transformed as desired. Each species to be transformed may make use of specific regulatory sequences as appropriate for those particular species. Transformed plants have been selected and their phenotypic properties analyzed. The transgenic plants were assessed for characteristics such as increased tolerance to drought, altered biomass accumulation, yield, nutritional requirements such as minerals or micro-nutrients, biotic stress such as fungal, bacterial, or other such pathogen infection or attack or any other such physical or biochemical characteristic.

Example 6: Plant Transformation

Arabidopsis thaliana transgenic plants were made by flower dipping method into an *Agrobacterium* culture. Wild type plants were grown under standard conditions until they began flowering. The plant was inverted for 2 min into a solution of *Agrobacterium* culture. Plants were then bagged for two days to maintain humidity and then uncovered to continue growth and seed development. Mature seed was bulk harvested.

Transformed T1 plants were selected by germination and growth on MS plates containing 50 µg/ml kanamycin. Green, kanamycin resistant seedlings were identified after 2 weeks growth and transplanted to soil. Plants were bagged to ensure self fertilization and the T2 seed of each plant harvested separately. During growth of T1 plants leaf samples were harvested, DNA extracted and Southern analysis performed.

T2 seeds were analyzed for Kan^R segregation. From those lines that showed a 3:1 resistant phenotype surviving T2 plants were grown, bagged during seed set, and T3 seed harvested from each line. T3 seed was again used for Kan^R segregation analysis and those lines showing 100% Kan^R phenotype were selected as homozygous lines. Further analysis was done using T3 seed.

Transgenic *Brassica napus* plants were produced using *Agrobacterium* mediated transformation of cotyledon petiole tissue. Seeds were sterilized as follows. Seeds were wetted with 95% ethanol for a short period of time such as 15 seconds. Approximately 30 ml of sterilizing solution I was added (70% Javex , 100µl Tween20) and left for approximately 15 minutes. Solution I was removed and replaced with 30 ml of solution II (0.25% mercuric chloride, 100µl Tween20) and incubated for about 10 minutes. Seeds were rinsed with at least 500 ml double distilled sterile water and stored in a sterile dish. Seeds were germinated on plates of ¹/₂ MS medium, pH 5.8, supplemented with 1% sucrose and 0.7% agar. Fully expanded cotyledons were harvested and placed on Medium I (Murashige minimal organics (MMO), 3% sucrose, 4.5 mg/L benzyl adenine (BA), 0.7% phytoagar, pH5.8). An *Agrobacterium* culture containing the nucleic acid construct of interest was grown for 2 days in AB Minimal media. The cotyledon explants were dipped such that only the cut portion of the petiole is contacted by the *Agrobacterium* solution. The explants were then embedded in Medium I and maintained for 5 days at 24°C, with 16,8 hr light dark cycles. Explants were transferred to Medium II (Medium I, 300 mg/L timentin,) for a further 7 days and then to Medium III (Medium II, 20 mg/L kanamycin). Any root or shoot tissue which had developed at this time was dissected away. Transfer explants to fresh plates of Medium III after 14 -21 days. When regenerated shoot tissue developed the regenerated tissue was transferred to Medium IV (MMO, 3% sucrose, 1.0% phytoagar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin). Once healthy shoot tissue developed shoot tissue dissected from any callus tissue was dipped in 10X IBA and transferred to Medium V (Murashige and Skooge (MS), 3% sucrose, 0.2 mg/L indole butyric acid (IBA), 0.7% agar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin) for rooting. Healthy plantlets were transferred to soil.

Transgenic *Glycine max*, *Zea maize* and cotton can be produced using *Agrobacterium*-based methods which are known to one of skill in the art. Alternatively one can use a particle or non-particle biolistic bombardment transformation method. An example of non-particle biolistic transformation is given in U.S. Patent Application 20010026941. Viable plants are propagated and homozygous lines are generated. Plants

are tested for the presence of drought tolerance, physiological and biochemical phenotypes as described elsewhere.

The following table identifies the constructs and the species which they have been transformed.

5

Table 11.

SEQ ID NO:	SEQ	Species Transformed	
SEQ ID NO:4	pBI121-35S-anti-AtFTA	Arabidopsis thaliana	
SEQ ID NO:40	pBI121-35S-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:41	pBI121-rd29A-anti-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:42	pBI121-35S-DA-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:43	pBI121-RD29A-DA-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:44	MuA-anti-GmFTA		Glycine max
SEQ ID NO:45	RD29A-anti-GmFTA		Glycine max
SEQ ID NO:46	MuA-HP-GmFTA-Nos-Term		Glycine max
SEQ ID NO:47	RD29AP-HP-GmFTA-Nos-Term		Glycine max
SEQ ID NO:48	pBI121-35S-Anti-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:49	pBI121-RD29AP-Anti-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:50	pBI121-35S-HP-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:51	pBI121-RD29AP-HP-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:52	pBI121-35S-AtFTB	Arabidopsis thaliana	
SEQ ID NO:53	MuA-anti-GmFTB-Nos-Term		Glycine max
SEQ ID NO:54	RD29AP-anti-GmFTB-Nos-Term		Glycine max
SEQ ID NO:55	MuA-HP-GmFTB-Nos-Term		Glycine max
SEQ ID NO:56	RD29AP-HP-GmFTB-Nos-Term		Glycine max
SEQ ID NO:57	MuA-anti-Zea maizeFTB-Nos-Term		Zea maize
SEQ ID NO:58	MuA-HP-Zea maizeFTB-Nos-Term		Zea maize

Non-limiting examples of vector constructs suitable for plant transformation are given in SEQ ID NO: 4, 40-58.

SEQ ID NO:4

10 *gtttaccgcgccaatatatcctgtcaaactgatagtttaactgaaggcgggaaacgacaatctgatcatg
agcggagaattaaggagtcacgttatgacccccgccgatgacgcgggacaagccgttttacgtttggaact*

gacagaaccgcaacggtgaaggagccactcagccgcgggtttctggagtttaatgagctaagcacatacgtc
 agaaaccattattgcggttcaaaagtcgcctaaggtcactatcagctagcaaatatttctgtcaaaaatg
 ctccactgacgttccataaattccccctcggtatccaatttagagttctcatattcactctcaatccaaaataatc
 5 tgcaccggatctggatcggttctgcattgaacaagatggattgcacgcagggttctccggccggttgggtg
 gagaggctattcggtatgactgggcacaacagacaaatcggtctctgatgcgcgcgtgttccggctgtca
 ggcaggggcccgggttctttttgtcaagaccgacctgtccggtgcctgaatgaactgcaggacgaggca
 gcgcggctatcggtggtggccacgacggggttcttgcgcagctgtgctcgacgttgtcactgaaagcggga
 agggactggctgctattgggcgaagtgcggggcgaggatctctgtcatctcaacttgccttgcgcgagaaa
 gtatccatcatggctgatgcaatgoggcggtgcatacgttgatccggctacctgccattcgaccaccaa
 10 gcgaacatcgcatcgagcgagcacgtactcggatggaagccggtcttgcgatcaggatgatctggacgaa
 gagcatcaggggtcgcgcacgacgaactgttgcgacgggtcaaggcgcgcgatgcgcgacggcgatgatctc
 gtcgtgacccatggcgatgcctgcttgcgaatatcatggtggaaaatggcgcgttttctggattcatcgac
 tgtggccggctgggtgtggcggaaccgtatcaggacatagcgttggctaccogtgcatttgcgtaagagctt
 ggcggcgaaatgggtgacgcgttctctgtgctttacggtatgcgcgcctcccgattgcgcgcctcgccttc
 15 tatcgcttcttgcagagttctcttgacgggactctgggttcgaaatgaccgaccaagcgacgcccaccc
 tgccatcacgagatttgcattccaccgcgcgttctctatgaaaggttgggttgcggaatcggtttccgggacg
 ccggtggtgatctctccagcgoggggtatctcatgctggagttcttgcgccacggggtctctgcggaacagg
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10

SEQ ID NO:4 is the nucleic acid sequence of pBI121-antisense-FTA vector
 construct used to transform *Arabidopsis* plants. Italicized sequences are the right and left
 border repeats (1-24, 5226-5230). Underlined sequence is the 35S promoter (2515-3318).
 Bold sequence is the anti-sense Farnesyl transferase alpha sequence (3334-4317).

15 SEQ ID NO:40

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 15 aaagaatcctggattagtgtatccaagtgttctcagtcgttgaatgttctatcccgacagattgcttc
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 (Underlined Seq: 35S promoter; Bold: AtFTA)

SEQ ID NO:41

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(Underlined Seq: RD29A promoter; Bold: Anti-sense-A1FTA)

50 SEQ ID NO:42

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 55 GGTAACAAGAAAGGGATCTTCACTCGCGACCGCAAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGG
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 acaccacaatatatcctgccca
 15 (Underlined Seq: 35S promoter; Bold: AtFTA anti-sense sequence separated by GUS
 Seq.)

SEQ ID NO: 43

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 45 cattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttgtttacaccacaataatctcctgcc
 a

(Underlined Seq: RD29A promoter; Bold: AtFTA anti-sense sequence, separated by GUS Seq.)

50 SEQ ID NO:44

GAATTCAAATTTTTCGCCAGTTCTAAATATCCGGAAACCTCTTGGGATGCCATTGCCCATCTATCTGTAATT
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 55 CCAACTATCCATCGCAAGACCATTGCTCTATATAAGAAAGTTAATATCATTTCGAGTGGCCACGCTGAGCTC
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 5 cccgaattatacatttaatacgcgatagaaaacaaaatatagcgcgcaaactaggataaattatcgcgcgcg
 gtgtcatctatgttactagatcggaattc

(Underlined MuA Promoter; Bold: *Glycine max* anti-FTA; lower case: NOS terminator Seq.)

SEQ ID NO:45

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 GGCCAATAGACATGGACCGACTACTAATAATAGTAAGTTACATTTTAGGATGGAATAAATATCATACCGACA
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(Underlined RD29A Promoter; Bold: *Glycine max* anti-*Glycine max* FTA; lower case: NOS terminator Seq.)

SEQ ID NO:46

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(Underlined: *Glycine max* FTA Anti-Sense section; Bold: MuA Promoter; Italics: *Glycine max* FTA Sense section; lower case: NOS terminator Seq.)

5

SEQ ID NO:47

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(Bold lower case: RD29A Promoter; Underline, Upper case: Antisense GmFTA; Upper case: Sense GmFTA; lower case: NOS terminator)

40 SEQ ID NO:48

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SEQ ID NO:49

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SEQ ID NO:50

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(Underlined: 35S promoter; Bold: Sense AtFTB)

40 SEQ ID NO:53

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(Upper Case: MuA Promoter; Underlined: Antisense GmFTB; Lower case: NOS

5 terminator)

SEQ ID NO:54

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 40 cgcgcggtgtcatctatgttactagatcggaattc

(Upper Case: RD29A Promoter; Underlined: Antisense GmFTB; Lower case: NOS)
 terminator

SEQ ID NO:55

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 60 CACCTTCATCATGCATCCTGAATCCACCATTGGTTGCTTCATCCGCCGAGAAACCCATACAGTTTATCTC
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 15 atatagcgcgcaaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcggggaattc
 (Upper Case: MuA Promoter; Underlined: Antisense GmFTB; Bold: Sense GmFTB;
 Lower case: NOS terminator)

SEQ ID NO:56

20 GGAGCCATAGATGCAATTCAATCAAACCTGAAATTTCTGCAAGAATCTCAAACACGGAGATCTCAAAGTTTGA
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 60 (Upper Case: RD29A Promoter; Underlined: Antisense GmFTB; Bold: Sense GmFTB;
 Lower case: NOS terminator)

SEQ ID NO:57

5 GAATTCAAATTTTTTCGCCAGTTCTAAATATCCGGAACCTCTTGGGATGCCATTGCCATCTATCTGTAATT
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 30 (Upper Case: MuA Promoter; Underlined: Antisense *Zea maize*-FTB; Lower case: NOS
 terminator).

SEQ ID NO:58

35 GAATTCAAATTTTTTCGCCAGTTCTAAATATCCGGAACCTCTTGGGATGCCATTGCCATCTATCTGTAATT
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 TGAACCATCCAGTAGCATAGCCAAGGGCGATTGGCATCTAGAACATGAAAGGCTGGTCCCATATGCCTCAGC
 CCAGGCGTCAGATACTCGATATGCTGATCAGCCACAGCTCTAGCATGATGGATTTTCGTGTTGGGCGCGGCC
 55 CCGAAGAGGGAGCGGTAGATGTGCGCAACCTGGCCTCCACCTTCATCTGCTCCACCTGCGTCACCGTGAGC
 CTCGGTAGGTCTGGGATCCGCCgagatccGCTGGGGAGCCTTATGCTGAAGCACATGGTGGGTATACATTCTGT
 GGATTGGCTGCTTTGATCCTGCTTAATGAGGCAGAGAAAGTTGACTTGCTTAGTTTGATTGGCTGGGTGGCT
 TTTCGTCAAGGAGTGGAATGCGGATTTCAAGGACGAACTAATAAATTGGTTGATGGTTGCTACTCCTTTTGG
 CAGGGAGCTGCCATTGCTTTACACAAAAGTTAATTACGATTGTTGATAAGCAATTGAGGTCCTCGTATTCC
 60 TGCAAAAGGCCATCAGGAGAGGATGCCTGCAGCACCAGTTCATATGGGTGCACCGCAATAAGTCTTCCTCT
 GCTGTGGACTATGCGAAGTTTGGATTGATTTTATACAACAGAGCAACCAAATTGGCCCACTCTTCATAAC

ATTGCCCTGCAACAATACATCCTACTTTGTTCTCAGGTACTAGAGGGAGGCTTGAGGGATAAGCCTGGAAAG
AACAGAGATCACTATCATTGCTACTGCCTCAGTGGCCTCGCAGTTAGCCAGTACAGTGCCATGACTGAT
ACTGGTTTCGTGCCCATTACCTCAGCATGTGCTTGGACCGTACTCTAATTTGCTGGAGCCAATCCATCCaagc
5 ttgaatttccccgatcgttcaaacatttggcaataaagtttcttaagattgaatcctgttgccggtcttgcg
atgattatcatataatttctgttggaattacgttaagcatgtaataattaacatgtaatgcatgacgttattt
atgagatgggtttttatgattagagtcgccgaattatacatttaatacgcgatagaaaacaaatatagcgc
gcaactaggataaattatcgcgcggtgtcatctatgttactagatcggaagctt
(Upper Case: MuA Promoter; Underlined: Antisense *Zea maize*-FTB; Bold: Sense *Zea*
maize-FTB; Lower case: NOS terminator)

10

Example 7: PCR Analysis of Putative Transgenic Plants

To verify that the putative transgenic plants carried the gene of interest PCR
analysis was performed. Genomic DNA was isolated and PCR run according to standard
protocols and conditions which are known to one of skill in the art. A typical reaction was
15 performed in a volume of 25 µl and primer pairs used were dependent on the gene and
promoter combination of the particular construct (Table 12).

Putative transgenic *Brassica napus* plants were screened using the primer
combinations detailed in the table below. A representative gel showing PCR analysis
results is shown in Figure 15 which represents transgenic plants carrying the pRD29A-
20 anti-FTA construct. Transformants were confirmed in an analogous manner for each
species and construct transformation done.

Table 12.

Construct Name	Primer Name	Primer Sequence (5'-3')
35S-antiFTA	SEQ ID NO:10	GCCGACAGTGGTCCCAAAGATGG
	SEQ ID NO:11	AAAGGATCCTCAAATTGCTGCCACTGTAAT
rd29A-antiFTA	SEQ ID NO:12	AAACCCGGGATGAATTTGACGACGAGAACGTG
	SEQ ID NO:13	GCAAGACCGGCAACAGGA
rd29B-antiFTA	SEQ ID NO:14	TTTAAGCTTGACAGAAACAGTCAGCGAGAC
	SEQ ID NO:11	AAACCCGGGATGAATTTGACGACGAGAACGTG
35S-DA-FTA	SEQ ID NO:15	GCTCTTCTCCATGCCCCA
	SEQ ID NO:13	GCAAGACCGGCAACAGGA
rd29A-DA-FTA	SEQ ID NO:16	TTTAAGCTTGGAGCCATAGATGCAATTCAA
	SEQ ID NO:17	CGGGCATTAGGAGGATGGGAA
35S-HP-FTB	SEQ ID NO:10	GCCGACAGTGGTCCCAAAGATGG
	SEQ ID NO:18	GTCCGGAATTCCCGGGTC
rd29A-HP-FTB	SEQ ID NO:16	TTTAAGCTTGGAGCCATAGATGCAATTCAA
	SEQ ID NO:18	GTCCGGAATTCCCGGGTC

25

Example 8: Southern Analysis

Genomic Southern analysis of anti-FTA transgenic *Arabidopsis thaliana*. The
numbers indicate the line numbers. Five micrograms of genomic DNA of T1 plants was
30 digested with *Hind*III (a unique site in the T-DNA plasmid) and separated in a 0.8%

agarose gel. The NPTII coding region was used as the probe for radio-labeling. Figure 2 shows a typical result from Southern analysis indicating the presence of the transgene.

Example 9: Northern blots of antisense FTA lines

RNA was isolated from developing leaf tissue of five 35S-anti-FTA *Arabidopsis thaliana* lines (T3 plants). The blot was first probed with P³² labeled, single-stranded sense transcript of FTA (Figure 3 panel A) which detects antisense transcript, then stripped and re-probed with the single-stranded anti-sense transcript of FTA (Figure 3 panel B) that detects the sense transcript. Figure 3 panel C shows the ethidium bromide stained gel for the blot. Approximately 5 µg of total RNA was loaded into each lane. Figure 3 indicates the accumulation of the transgene anti-sense transcript and a reduction in the sense transcript in transgenic plants.

Example 10: Western blot antisense FTA lines with Anti-FT-α antibodies.

The antibodies produced according to the methods of Example 19 were used to analyze protein extracts from transgenic plants on western blots. Lane 1 of Figure 4 is a molecular weight standard, lane 2 purified FTA protein, lanes 3-10 are protein extracts from the ERA1 mutant, wild type, and 4 lines of transgenic *Arabidopsis thaliana*. Figure 4 illustrates the reduction of detectable FTA protein in transgenic lines.

Example 11: ABA sensitivity of transgenic seedlings.

Seeds of wild type Columbia, era1-2 and T3 homozygous seeds of two antisense, drought tolerant lines of 35S-antisense-FTA were plated on minimum medium (1/2 MS) supplemented with no ABA (A), 0.3 µM (B), 0.5 µM (C) or 1.0 µM ABA (D). Plates were chilled for 3 days in 4 °C in the dark, and incubated for 11 days at 22 °C with 24 hour continuous light. era1 and transgenic lines were more inhibited in germination than wild type plants. Results are shown in Figure 5.

Twelve day old seedling phenotypes of wild type Columbia, era1-2 and two drought tolerant 35S-antisense-FTA lines (9.9 & 21.2) in minimum medium without (A) or with (B) 1 µM ABA. Figure 6 shows the reduced root growth and development of era1 and transgenic lines relative to wild type plants. The 35S-antisense-FTA lines show reduced root growth, similar to the era1 mutant, in response to ABA.

A transgenic *Brassica napus* line carrying the 35S-antisense-FTA construct was assessed for ABA sensitivity. At about 10µm an effect was observed showing reduced

seedling development and vigor at the cotyledon and first leaf stage, thereby indicating an increased sensitivity to ABA

ABA sensitivity is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the methods above. The ABA

5 concentration used varies depending upon the species under examination.

Example 12: Drought Experiment

To assess the response of plants under water stress or drought one can expose plants to various situations. For example, the plant can be removed from soil or media and placed on paper towel for a period of time, such as 4 hours, then returned to a plate to
10 continue growth and development. Survival and vigour can be assessed.

Alternatively one can impose a water stress in such a way as to more closely resemble a field situation by withholding water for a period of time, such as up to 6 days. Plants were grown five plants per four inch pot, in a replicated water-stress experiment. All pots were filled with equal amounts of homogeneous premixed and wetted soil.
15 Growth conditions were 16 hour daylight ($150\text{--}200\ \mu\text{mol}/\text{m}^2/\text{s}$) at $22\ ^\circ\text{C}$ and 70% relative humidity. On the day that the first flower opened drought treatment was initiated first by equalizing the soil water content in each pot on a weight basis and then cessation of watering. At the end of the water stress treatment plants were typically either harvested for biomass data or re-watered to complete the life cycle and determination of biomass and
20 yield data. Physiological parameters have been assessed under stressed and optimal conditions, for example, shoot and root biomass accumulation, soil water content, water loss alone or as a function of parameters such as biomass, seed yield, and leaf number and leaf area. Figure 7 shows photographs of wild type Columbia (A) and four 35S-antisense-FTA transgenic *Arabidopsis thaliana* lines (B,C,D,E) after 8 days of water stress
25 treatment. The control plant is visibly stressed and less healthy. This experiment has been conducted on transgenic lines containing vectors described by SEQ ID NO: 4, 40-58.

Drought or water stress tolerance is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the described methods.

Example 13: Analysis of Water Loss in *Arabidopsis thaliana* pRD29A-DA-FTA lines during drought stress

30

Plants were grown 5 plants per 4 inch pot and 6 pots per line. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 12. Pots were weighed daily and at the end of the 7 day drought treatment all plants were

harvested for shoot fresh weight and dry weight determinations. Figure 10 shows the water loss on a per shoot dry weight basis at 4 days of water stress treatment. Of the 31 lines examined in this experiment 25 showed lower water loss relative to the Columbia wild type, 22 of which were statistically significant. All lines had been assessed for ABA sensitivity as described in Example 6, increased ABA sensitivity (ABA^S) also correlated with a decreased water loss during drought treatment. Those lines determined to have wild type ABA sensitivity (ABA^{WT}) were the same 6 lines (lines 2, 36, 69, 29, 24, 21) that did not show a reduced water loss compared to wild type.

The above experiment was repeated using two ABA^S lines, one ABA^{WT} line and a Columbia control. Plants were harvested after 2, 4 and 6 days of water stress treatment for shoot dry weight determinations. ABA^S transgenics had greater leaf and shoot biomass, greater soil water contents and lower water loss per shoot dry weight when compared to the ABA^{WT} or Columbia controls. Results were consistent at all three harvest stages.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has also been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar water stress tolerant trends observed. Soil water loss is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the described methods.

Example 14: Analysis of Shoot Fresh Weight in *Arabidopsis thaliana* pRD29A-DA-FTA lines during drought stress

Plants were grown 5 plants per 4 inch pot and 8 pots per line. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 12. Plants were re-watered after 6 days drought treatment and allowed to recover for an additional 6 days. Plants were harvested and shoot fresh weights determined. Figure 11 shows the shoot fresh weights. This experiment consisted of 25 transgenic lines, 2 of which are ABA^{WT} (line 2 and 69) and a Columbia wild type control. All 23 ABA^S transgenic lines had statistically significant greater shoot fresh weights, on average 44% greater.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 15: Analysis of seed yield in *Arabidopsis thaliana* pRD29A-DA-FTA lines during drought stress and under optimal conditions

Plants were grown 1 plant per 4 inch pot. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 12. Plants were re-watered after 6 days drought treatment and allowed to grow to maturity. The optimal group was not exposed to the drought treatment.

Yield analysis indicates that although drought treatment results in decreased yields, the transgenics do not suffer as severely as controls and maintain a productivity advantage (Figure 12) as shown previously in Experiment 14. Comparison of the yields produced by the ABA^S transgenics versus the control plants show that a 15% greater yield was obtained under optimal conditions and a 20% increase under drought conditions. In the drought treatment group 8 of 9 transgenic lines showed greater yield than controls. Expression of yield of each line obtained under drought treatment as a percentage of its performance under optimum conditions indicates that 8 of 9 ABA^S lines outperformed the control line while 4 of 9 out performed the ABA^{WT} controls.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 16: Analysis of vegetative growth in *Arabidopsis thaliana* pRD29A-DA-FTA lines under optimum growth conditions

Plants were grown 1 plant per 3 inch pot and 8 pots per line. Plants were harvested at three stages and fresh weights determined. Vegetative stage was defined as 14 day old seedlings, bolting stage as the appearance of first flower (19-21 day seedlings) and mid-flowering as 6 days from first flower. At each of the above stages respectively 7, 8 and 10 of the 10 ABA^S transgenic lines tested showed statistically greater shoot fresh weight biomass than the control plants (Figure 13). One Columbia line and an ABA^{WT} (line 2) line were used as the control group. Additionally, there was a statistically significant trend for the transgenic lines to have an increased number of rosette leaves.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 17: Analysis of *Arabidopsis thaliana* pRD29A-DA-FTA lines under drought treatment and biotic stress

Plants were grown 1 plant per 4 inch pot and 8 pots. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 12. Plants were re-watered after 7 days drought treatment and allowed to grow to maturity. One Columbian control line (col) and one transgenic line were evaluated. Analysis of seed yield indicated less than normal yields, approximately 12% of expected optimal yield. It was determined that the soil used contained a fungal contaminant that was responsible for the reduced yields as the biotic stress could be negated by sterilization of the soil prior to use. This biotic stress was less severe in the transgenic line compared to the control which had a yield 22% of the transgenic line. In the drought treatment groups of plants the biotic stress was reduced however, transgenics outperformed controls by nearly 4.5 fold (Figure 14).

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 18: Analysis of *Arabidopsis thaliana* pRD29A-DA-FTA lines for Stomatal number

The number of stomata on both the upper and lower surface of the leaf was assessed on two transgenic lines and a wild type Columbia control. Nail polish imprints were made of both upper and lower leaf surfaces of the fifth leaf, plants were at the early flowering stage. No differences in stoma density were observed.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 19: Production of polyclonal antibodies against FT-A and FT-B

The isolated *Arabidopsis thaliana* FT sequences were cloned into the *E. coli* expression vector derived from pET11D. To generate the Histidine tagged FT-B construct the *Arabidopsis thaliana* FT-B clone and pET vector were digested with *Bam*HI and ligated together. Restriction digests were performed to verify the orientation of the insert. To produce the FT-A construct the *Arabidopsis thaliana* FT-A clone and pET vector were digested with *Bam*HI and *Eco*RI and subsequently ligated together. The resultant plasmids

directed the expression of fusion proteins containing 6 consecutive histidine residues at the N-termini of AtFTA and AtFTB. The fusion proteins were expressed in the bacterial host BL21(DE3) and purified using Hi-Trap chelating chromatography as described by the manufacturer (Pharmacia). The soluble fraction of the crude bacterial extract containing the His-FT fusion proteins were loaded to a Hi-Trap column (1.5 cm x 2.0 cm), and the proteins eluted with a 200 ml linear gradient of 0.0 to 0.3 M imidazole in column buffer (25 mM Tris-HCl, pH 7.5, 1 mM DTT). Fractions containing purified His-FT proteins were pooled, desalted and concentrated with a Centriprep-30 concentrator (Amicon). All purification steps were carried out at 4 °C. To generate an antibody, the purified fusion protein was further separated by SDS/PAGE and the Coomassie stained band corresponding to the fusion protein was excised. Protein was eluted from the gel slice by electroelution and then emulsified in Ribi adjuvant (Ribi Immunochem) to a final volume of 1 ml. His-AtFTA or His-AtFTB (250 µg) were injected into a 3 kg New Zealand rabbit on day 1 and booster injections given on day 21 and day 35 with 200 µg of the protein. High-titer antisera were obtained one week after the final injection. These antibodies were used in the western analysis of example 10, Figure 4.

Example 20: Screening for related genes

The transgenic plants of the invention can be used to identify genes which interact with the genes of the present invention. One can make use of the transgenic plants of the invention to screen for related genes, for example, suppressors, enhancers or modulators of gene expression or activity can be identified through genetic screening protocols. By way of example, a mutant library can be generated using the transgenic plants of the invention as the genetic background. Various methods are available and would be known to one of skill in the art. For example, chemical mutagens such as EMS can be used to induce point mutations in the genome, fast neutron irradiation of seeds can result in deletion mutations, T-DNA libraries can be produced that inactivate genes through insertional effects or activation tagging methods can be used to produce libraries with up-regulated genes. Analysis of these types of libraries can identify genes which rescue or modulate the phenotypes observed in the transgenic plants of the present invention.

What is claimed is:

1. A method of producing a transgenic plant, wherein said plant has an increased tolerance to stress or delayed senescence compared to a wild type plant, comprising introducing into a plant cell a nucleic acid that inhibits farnesyl transferase alpha expression or activity to generate a transgenic cell; and regenerating a transgenic plant from said transgenic cell.
2. The method of claim 1, wherein said nucleic acid comprises an antisense nucleic acid sequence encoding farnesyl transferase alpha.
3. The method of claim 2, wherein said antisense nucleic acid comprises 20 or more consecutive nucleic acids complementary to SEQ ID NO: 1, 6 or 31.
4. The method of claim 2, wherein said antisense nucleic acid comprises SEQ ID NO: 2, 3, 29, or 32.
5. The method of claim 1, wherein said nucleic acid is selected from the group consisting of SEQ ID NO: 4, 40-46 or 47.
6. The method of claim 2, wherein said antisense nucleic acid is operably linked to a promotor.
7. The method of claim 6, wherein said promotor is selected from the group consisting of a constitutive promotor, an ABA inducible promotor, tissue specific promoters or a guard cell-specific promotor
8. The method of claim 1, wherein the nucleic acid is an inhibitor of farnesylation or geranylgeranylation.
9. The method of claim 1, wherein said nucleic acid comprises a nucleic acid sequence encoding farnesyl transferase alpha.

10. The method of claim 9, wherein said nucleic acid comprises SEQ ID NO: 1, 6 or 31.
11. A method of producing a transgenic plant, wherein said plant has increased tolerance to stress or delayed senescence, comprising introducing into a plant cell a nucleic acid that inhibits the farnesyl transferase expression or activity to generate a transgenic cell, wherein said nucleic acid is a nucleic acid comprising an antisense nucleic acid sequence encoding farnesyl transferase alpha; and regenerating a transgenic plant from said transgenic cell.
12. The transgenic plant produced by any one of the methods of claims 1 or 11
13. The seed produced by the transgenic plant of claim 12, wherein said seed produces a plant that has increased tolerance to stress or delayed senescence.
14. An isolated polypeptide comprising the mature form of an amino acid sequenced selected from the group consisting of SEQ ID NO: 5, 7, 9, 33, 36 or 39.
15. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, 7, 9, 33, 36 or 39.
16. An isolated polypeptide comprising an amino acid sequence which is at least 85% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 5, 7 or 9.
17. An isolated polypeptide comprising an amino acid sequence which is at least 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 33, 36 or 39
18. The polypeptide of claim 15, wherein said polypeptide has farnesyl transferase activity.

19. An isolated polypeptide, wherein the polypeptide comprises an amino acid sequence comprising one or more conservative substitutions in the amino acid sequence selected from the group consisting of SEQ ID NO: 5, 7, 9, 33, 36, or 39.
20. The polypeptide of claim 14, wherein said polypeptide is naturally occurring.
21. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 6, 8, 31, 34, or 37.
22. The nucleic acid molecule of claim 21, wherein the nucleic acid molecule is naturally occurring.
23. An isolated nucleic acid molecule encoding the mature form of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: SEQ ID NO: 5, 7, 9, 33, 36, or 39.
24. An isolated nucleic acid molecule, wherein said nucleic acid molecule hybridizes under stringent conditions to the nucleotide sequence selected from the group consisting of SEQ ID NO: SEQ ID NO: 1, 6, 8, 31, 34, or 37.
25. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 90% identical to the nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 6, or 8
26. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 99% identical to the nucleotide sequence selected from the group consisting of SEQ ID NO: 31, 34 or 37
27. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, 3, 29, 30, 32, 35 or 38.

28. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 90% identical to the nucleotide sequence selected from the group consisting of SEQ ID NO:2, 3, 29 or 30.
29. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 99% identical to the nucleotide sequence selected from the group consisting of SEQ ID NO: 32, 35 or 38.
30. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, 40-58.
31. A vector comprising the nucleic acid molecule of claim 21.
32. The vector of claim 31, further comprising a promoter operably linked to said nucleic acid molecule.
33. A cell comprising the vector of claim 32.
34. A vector comprising the nucleic acid molecule of claim 27.
35. The vector of claim 34, further comprising a promoter operably linked to said nucleic acid molecule.
36. A cell comprising the vector of claim 34.
37. An antibody that immunospecifically binds to the polypeptide of claim 14.
38. The antibody of claim 37, wherein the antibody is a monoclonal antibody.
39. The antibody of claim 37, wherein the antibody is a polyclonal antibody.

40. A method of identifying an agent that binds to the polypeptide of claim 14, the method comprising:
- (a) introducing said polypeptide to said agent; and
 - (b) determining whether said agent binds to said polypeptide.
41. The method of claim 40, wherein the agent is a farnesyl transferase inhibitor.
42. A method for identifying farnesyl transferase modulator, the method comprising:
- (a) providing a cell expressing the polypeptide of claim 14;
 - (b) contacting the cell with a candidate substance; and
 - (c) determining whether the substance alters farnesyl transferase activity;
- whereby, if an alteration observed in the presence of the substance is not observed when the cell is contacted with a composition in the absence of the substance, the substance is identified as a farnesyl transferase modulator.
43. A method for identifying an interacting gene of farnesyl transferase, the method comprising:
- a) providing the transgenic plant of claim 12;
 - b) creating a library of mutagenized plants from (a);
 - c) determining whether the mutagenized plant contains an altered phenotype;
- whereby, the mutagenized plant has altered the function of an interacting gene of farnesyl transferase which results in an altered phenotype from the transgenic plant of (a) to that of a wild type non-transgenic plant.
44. A plant, wherein a mutation has been introduced in the gene encoding farnesyl transferase, resulting in said plant displaying a decrease in farnesyl transferase activity and an increased tolerance to stress as compared to a wild type plant.

45. A method of producing a transgenic plant, wherein said plant has an increased tolerance to stress or delayed senescence compared to a wild type plant, comprising introducing into a plant cell a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 30, 35, 38 48-57 or 58 to generate a transgenic cell; and regenerating a transgenic plant from said transgenic cell.
46. A method of producing a transgenic plant, wherein said plant has an increased sensitivity to abscisic acid compared to a wild type plant, comprising introducing into a plant cell a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 30, 35, 38 48-57 or 58 to generate a transgenic cell; and regenerating a transgenic plant from said transgenic cell.

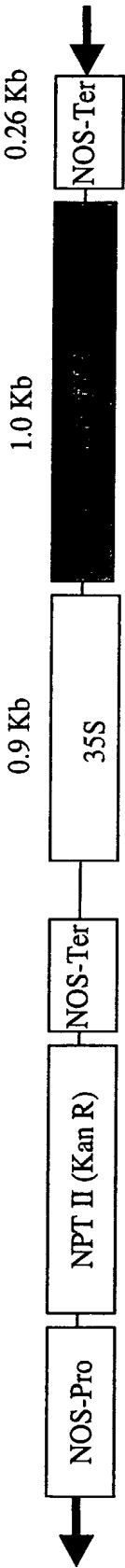


Figure 1

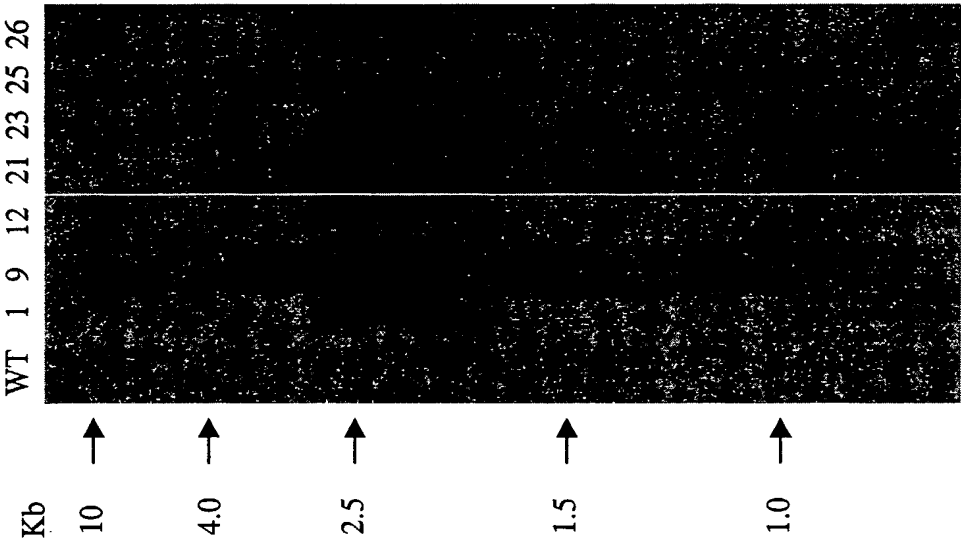


Figure 2

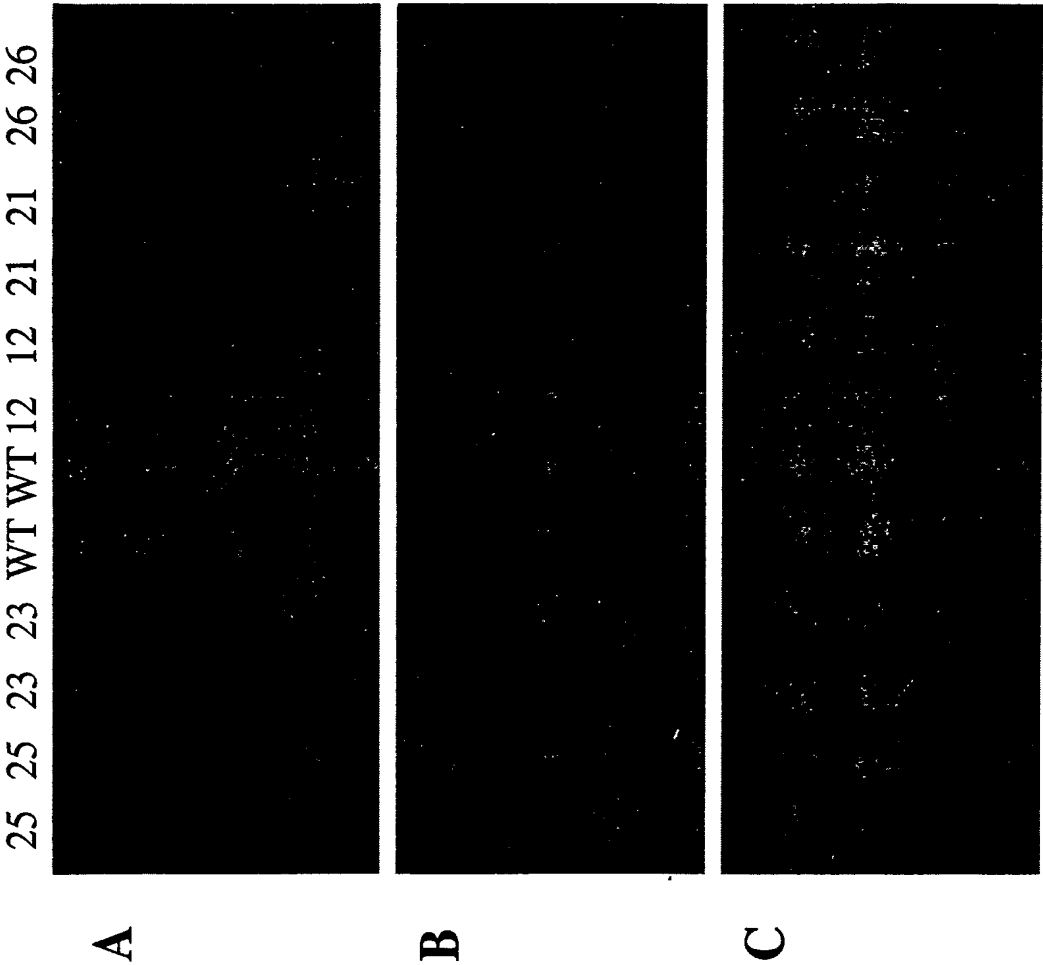


Figure 3

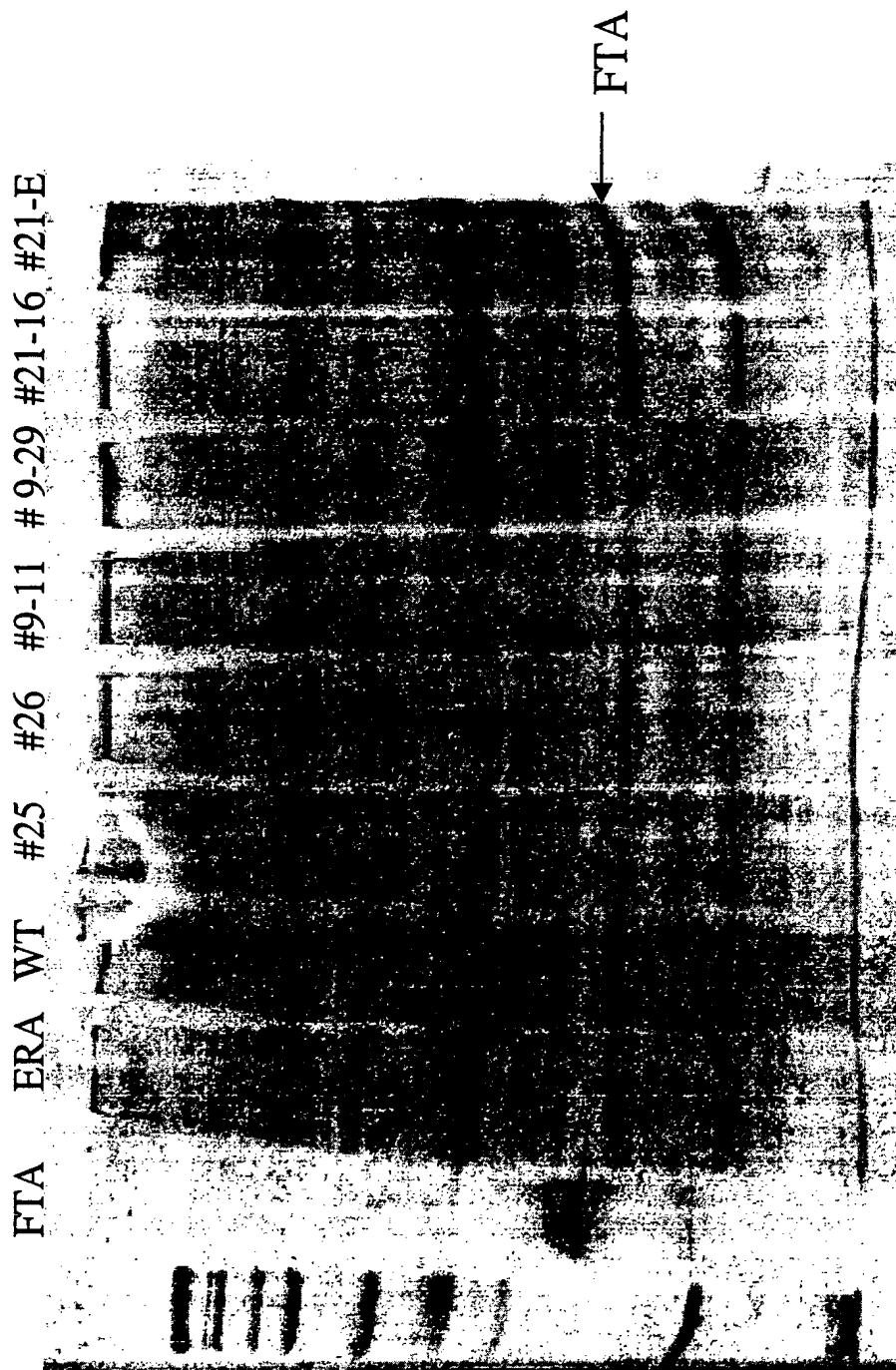
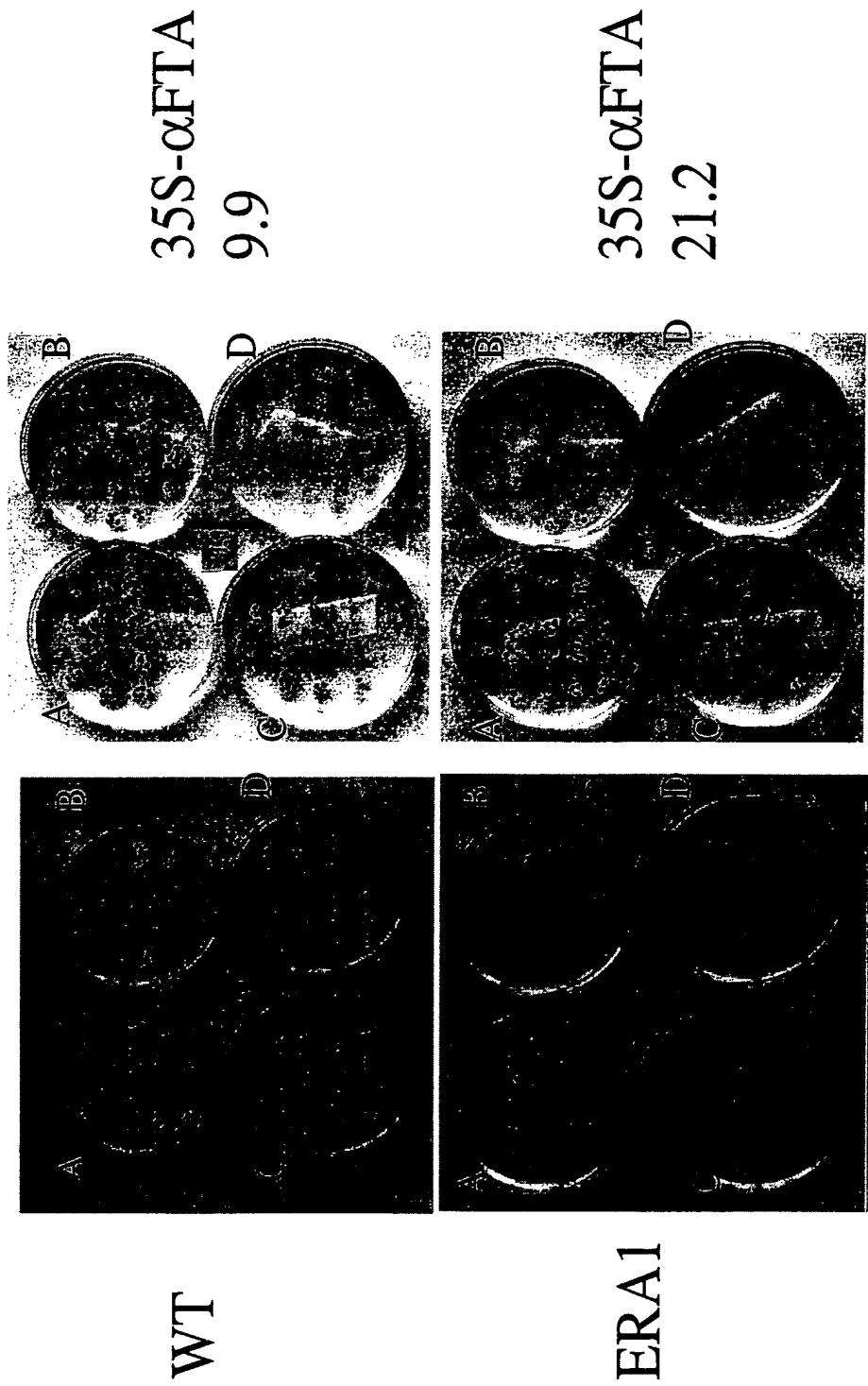


Figure 4



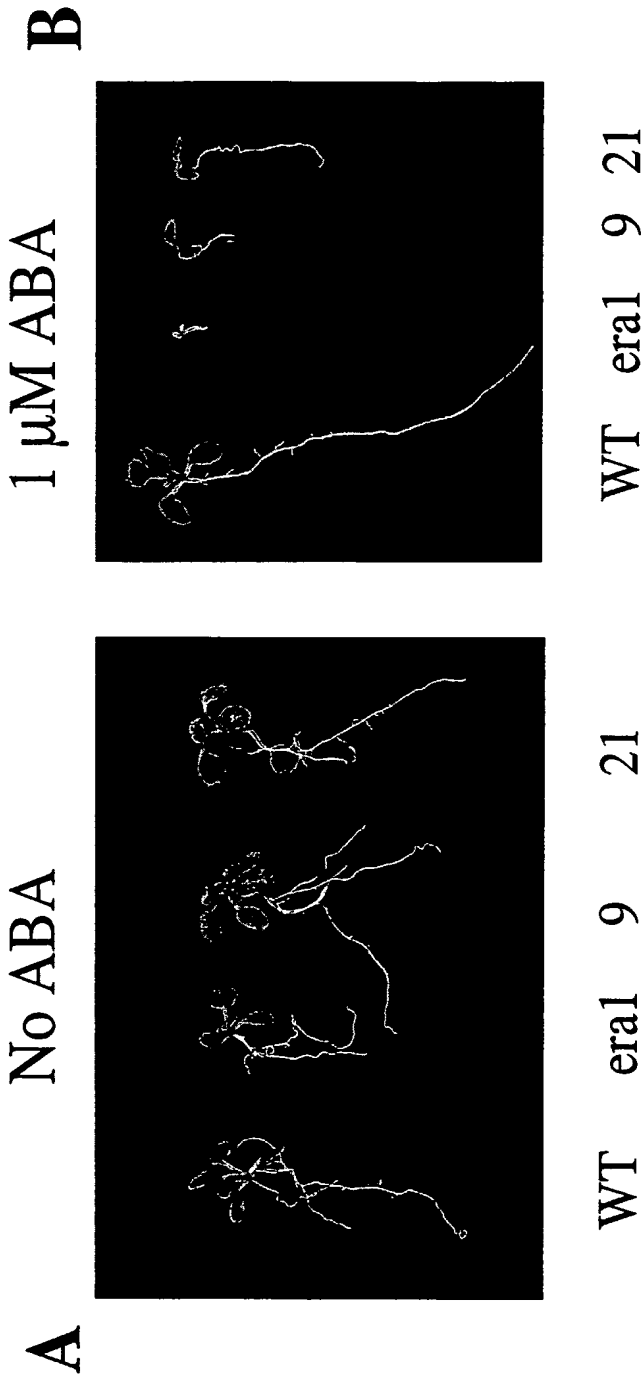


Figure 6

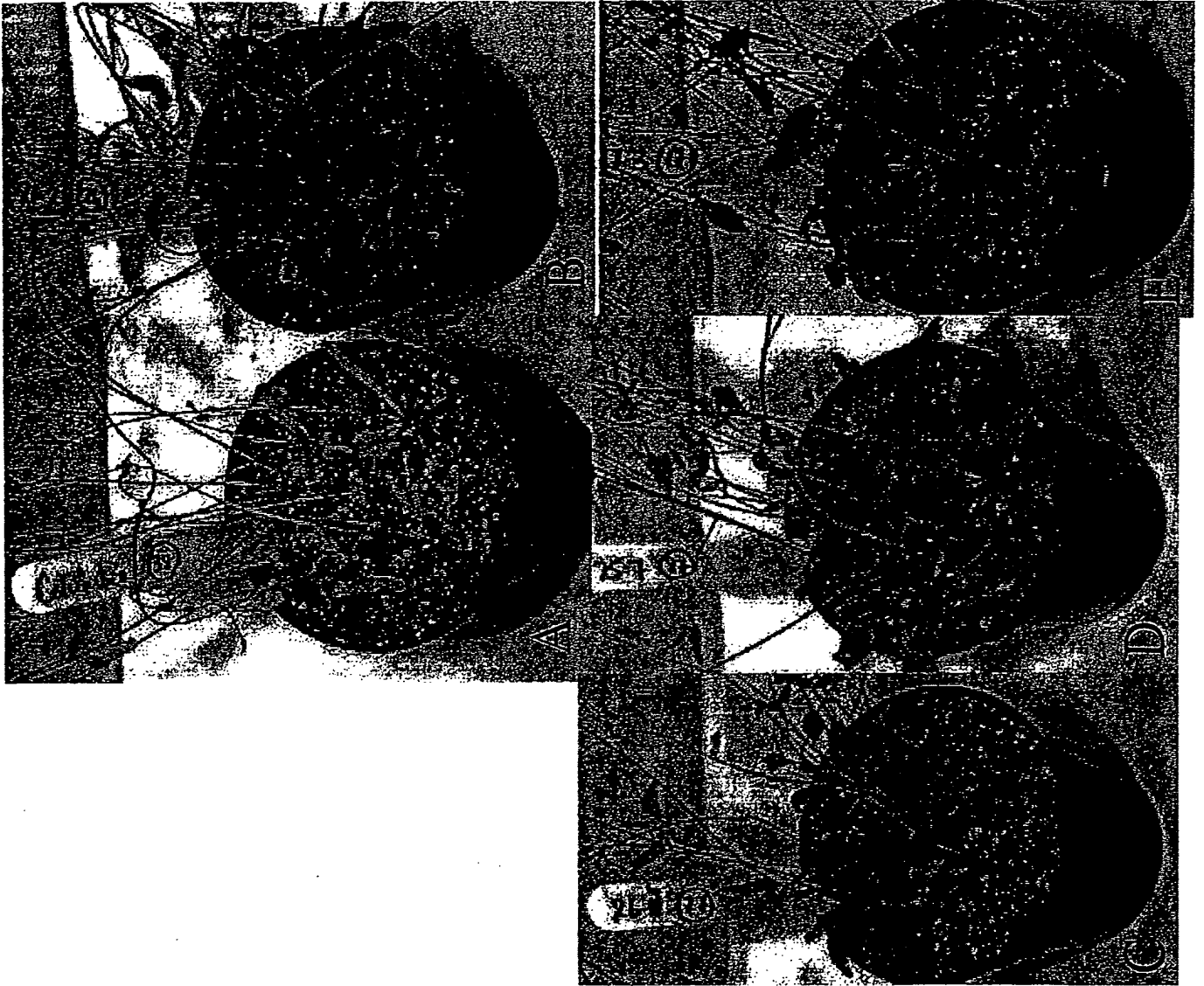


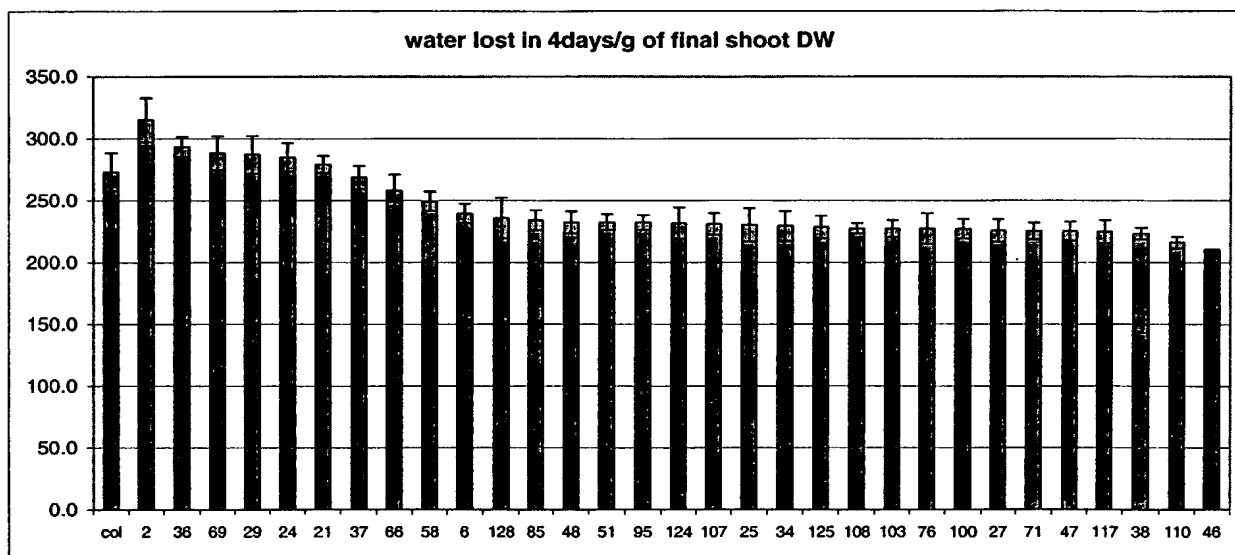
Figure 7.

DNA	Brassica napus	Arabidopsis thaliana	PPI Glycine max	Zea mays	Rice	Soy 1	Soy 2	Triticum	Tomato	Pea
Brassica napus	X									
Arabidopsis thaliana	89	X								
PPI Glycine max	61	55	X							
Zea mays	57	45	52	X						
Rice	55	46	54	63	X					
Soy 1	61	50	98	43	47	X				
Soy 2	61	50	99	41	46	99	X			
Triticum	58	45	52	56	66	43	41	X		
Tomato	65	53	63	44	51	52	49	41	X	
Pea	66	55	78	46	50	70	69	44	49	X
PROTEIN										
Brassica napus	X									
Arabidopsis thaliana	89	X								
PPI Glycine max	65	63	X							
Pea	61	61	77	X						
Tomato	60	59	57	58	X					
Rice	64	63	56	58	58	X				
Zea mays	61	56	58	57	56	75	X			
Soy 1	66	64	98	77	58	57	58	X		
Soy 2	66	64	98	78	58	57	58	99	X	
Triticum	61	60	57	59	60	80	73	58	58	X

Figure 8

DNA	Brassica napus	Arabidopsis thaliana	Wiggum	PPI Glycine max	Glycine max	PPI Zea maize	Zea maize	Pea	Tomato	Tobacco
Brassica napus	X									
Arabidopsis thaliana	88	X								
Wiggum	88	99	X							
PPI Glycine max	60	64	65	X						
Glycine max	60	64	65	99	X					
PPI Zea maize	38	54	59	63	63	X				
Zea maize	54	54	59	62	62	99	X			
Pea	65	57	45	78	77	56	56	X		
Tomato	68	62	52	70	70	64	64	51	X	
Tobacco	68	64	60	71	71	65	65	55	83	X
PROTEIN	Brassica napus	Arabidopsis thaliana	Wiggum	PPI Glycine max	Glycine max	PPI Zea maize	Zea maize	Pea	Tomato	Tobacco
Brassica napus	X									
Arabidopsis thaliana	84	X								
Wiggum	84	99	X							
PPI Glycine max	54	58	59	X						
Glycine max	53	58	58	99	X					
PPI Zea maize	52	50	52	58	58	X				
Zea maize	51	50	52	58	58	99	X			
Pea	58	56	57	78	78	56	56	X		
Tomato	60	62	55	63	63	58	58	62	X	
Tobacco	62	63	59	64	63	58	58	64	83	X

Figure 9

**Figure 10**

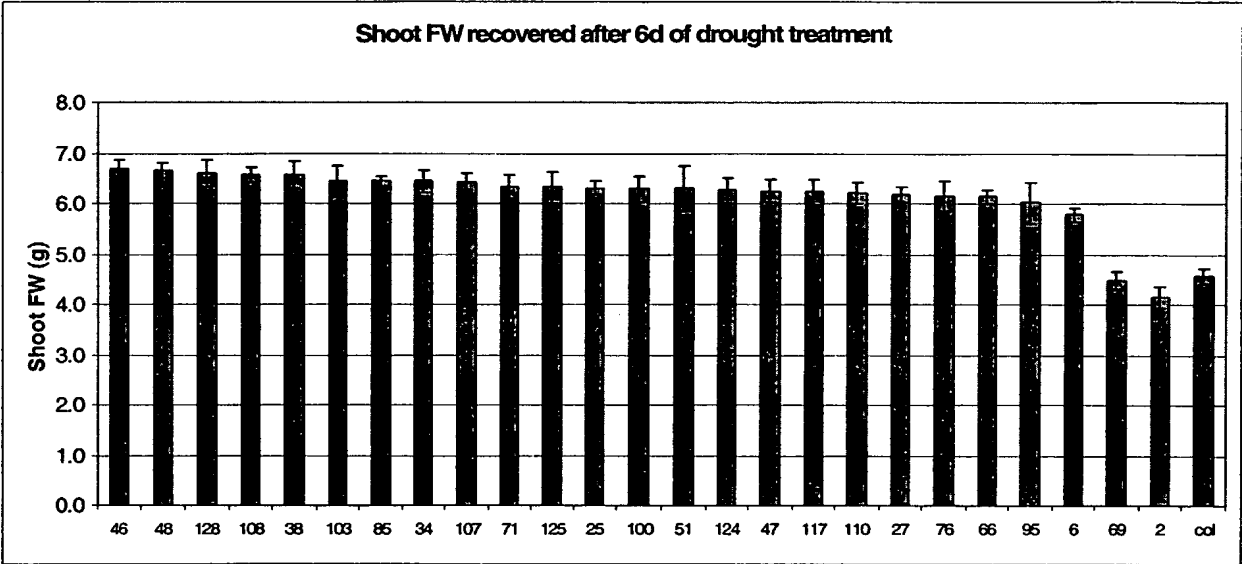


Figure 11

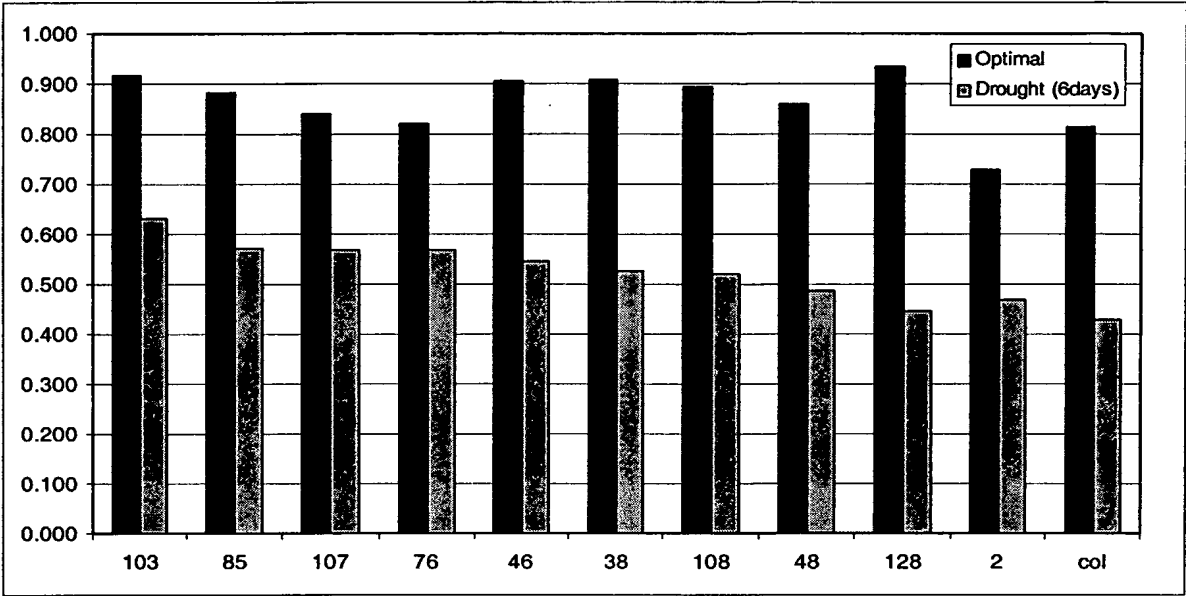


Figure 12

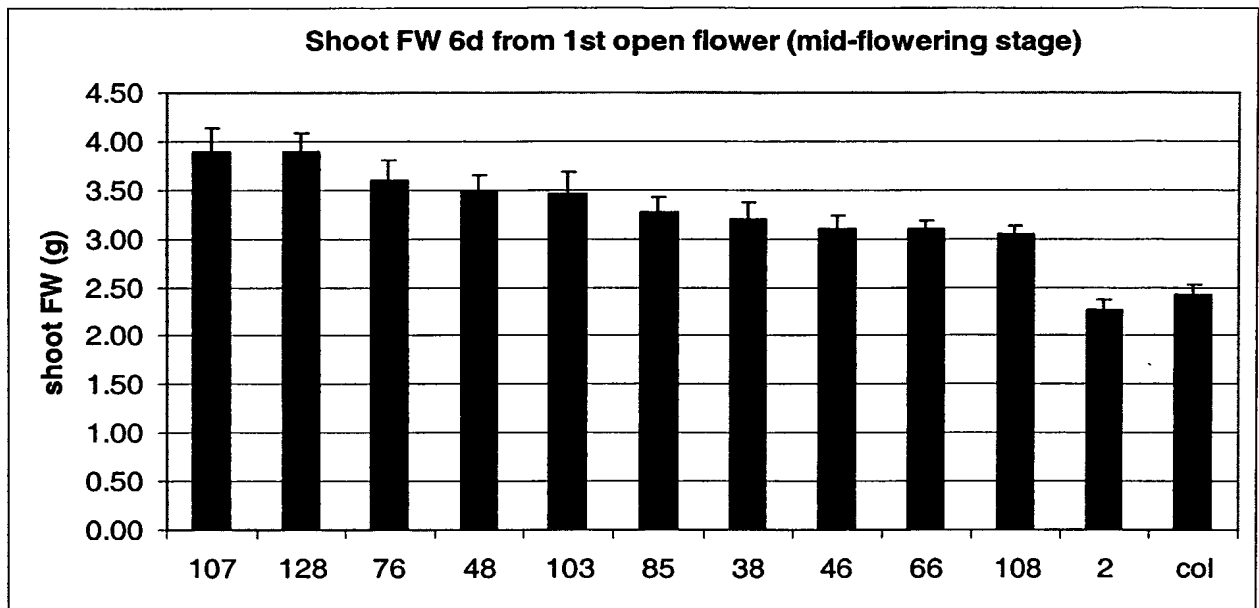
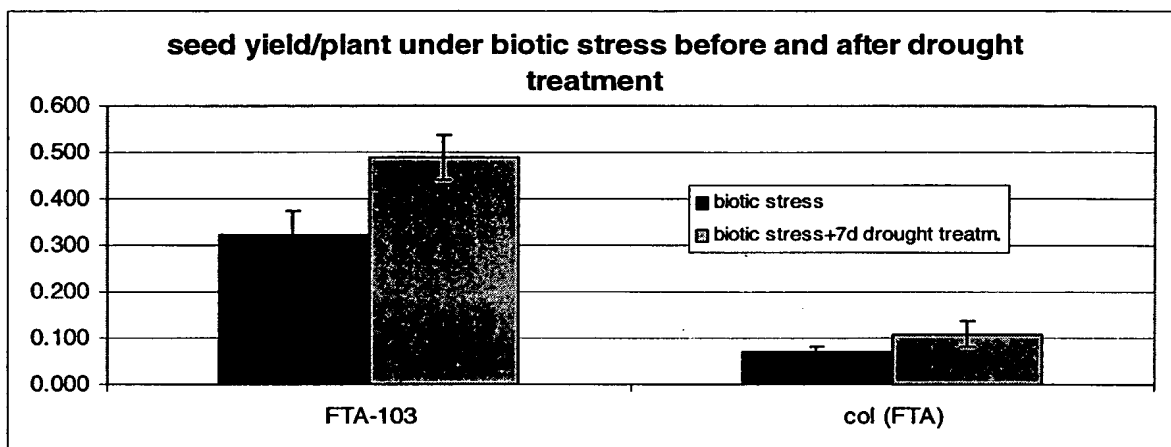


Figure 13

**Figure 14**

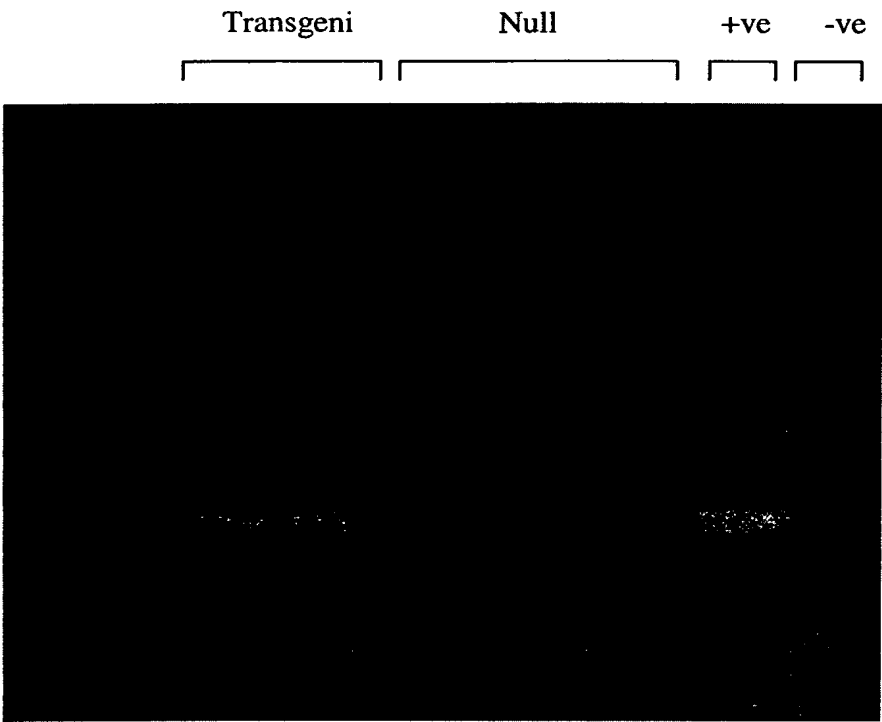


Figure 15